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(54) Biological Assays

(57) Biological assays using various constructions of biochips are disclosed to mirror in vivo situations. The biochip 50 comprises a microchannel 51 having a liquid outlet port 1, bubble release port 2 and a liquid outlet port 3 with an associated bubble release port 4. A multiplicity of tests can be performed often by coating the bore of the microchannel 50 with various adhesion mediating proteins or the use of chemoattractants. The assay assembly 60 comprises a syringe pump feeding the biochip 50. An inverted microscope 65, digital camera 66 and recorder 67 are provided. A sample liquid containing cells in suspension is injected slowly through the biochip and the effect of the assay recorded over a long period of time.

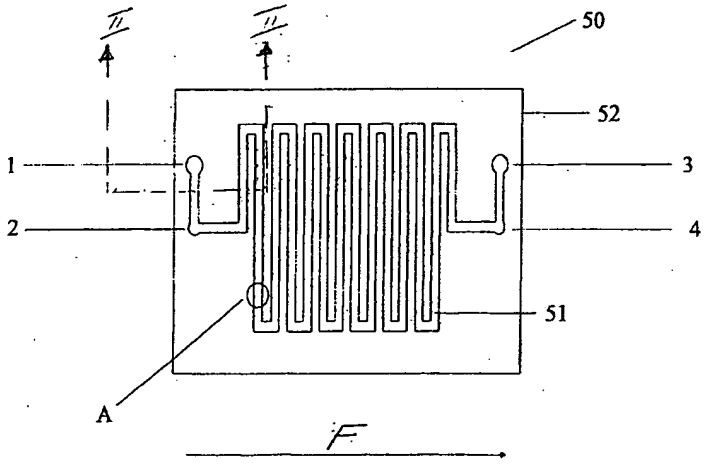

Fig.1

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Description

Introduction

[0001] The present invention relates to a biological assay and a biological assay**biochip**.

[0002] Biochemical, microbiological, chemical and many other assays are performed every day in laboratories. While a considerable amount of attention has naturally been placed on such biological cell assaying for humans, this is also becoming more important in the field of animal welfare and indeed plant production generally.

[0003] A rapidly advancing research area in biology is the study of cell receptor-ligand interactions resulting in cell-substratum and cell-cell adhesion followed by subsequent cell migration. The pre-requisite to transendothelial migration of certain cell lines into sites of infection is paramount to the study of inflammatory diseases. This can be briefly summarised as cell flow and rolling, tethering and activation of integrin receptors which is a key recognition step, attachment to the endothelial ligands via activated integrins and finally transendothelial migration or diapedesis. Unfortunately, to date, most of the assay techniques are not particularly successful for the study of these mechanisms. Currently, the majority of studies involving cell rolling and chemokine induced cellular arrest have utilised capillary systems wherein cell flow and shear stress are controlled utilising syringe pumps. Such observations are constrained by a number of factors. Firstly, the relative large (> 100 μm) size of the standard glass capillaries limits the physiological analogies to the proximal microvascular regions. Secondly, such studies can only be utilised to study single end-points and cannot be utilised to examine cell choices in migration. Thirdly, optical aberrations related to the spherical geometry of the glass capillary sections limit stage-related in situ (post-fixation) analysis of the intracellular structures (cytoskeleton and signalling molecules). Finally and most importantly, the usual observation periods lie between 5-30 minutes for rolling experiments. Longer studies are required to study subsequent crawling steps on endothelial and extracellular matrix ligands. In this regard, studies relating to the effects of chemokines have largely been limited to cellular arrest on adhesion receptor ligands and have not been extended to the study of cell crawling. For example, specific chemokines have been shown to induce rolling arrest with enhanced binding of lymphocytes to ICAM-1, otherwise known as CD54.

[0004] Presently accepted techniques for cell adhesion or binding assays involve the initial coating of a surface of a device with a substrate, typically a protein. Cells are deposited onto the substrate and allowed to settle. Following the settling of the cells, the device is heated to 37°C and is visually analysed using an inverted microscope , or alternatively it is subjected to a stand-alone heating stage and progression of cell binding can be checked at intervals with the inverted microscope. The duration of these assays may be varied depending on the cell line and choice of substratum. Following cell adhesion, free cells may be washed away and a subsequent cell count may be carried out.

[0005] Although these methods provide semi-quantitative information regarding a cell type's affinity for a particular substratum, there is no simple method for quantitative characterisation of binding or methods enabling a prolonged study of cell rolling, the ensuing capture by the substratum and subsequent attachment. Furthermore, direct studies of changes in cell morphology, cell growth and biochemical changes cannot be provided easily with these techniques since, determining the kinetics of attachment and resulting morphological changes requires multiple replicated experiments being analysed at different times.

[0006] US Patent Specification No. 5998160 (Berens et al) describes a static assay which unfortunately does not have any consideration of cell flow and rolling.

[0007] The ability of T-cells circulating in the bloodstream to adhere to the endothelium, switch to a motile phenotype and penetrate through the endothelial layer is recognised as a necessary requirement for the effective in vivo movement or as it is sometimes referred to, trafficking of specific lymphocyte sub-populations Motility assays are done in combination with attachment assays since following adhesion; cells are expected to switch to the motile phenotype. Motility assays are assessed by estimating the ratio of cells undergoing cytoskeletal rearrangements and the formation of uropods (extension of the trailing tail). One of the major disadvantages of this and the previous adhesion assays is the geometrical design (microscope slides and multiple well chambers) which does not at all resemble the in vivo situation.

[0008] The most commonly used cell transmigration assay is a modified "Boyden chamber" assay such as described in US Patent Specification No. 5578492 (Fedun et al). This involves assessing the crossing of a quantity of cells through a microporous membrane under the influence of a chemoattractant, recombinant or cell-derived. Here the diameter of the micropores are less than the diameter of the cells under investigation, such that the cells must deform themselves in order to squeeze through the pores thereby constructing an analogy to the transendothelial migration of cells in physiological circumstances. Once cells are deposited onto the membrane, the chamber can be incubated for intervals over time at a suitable temperature, usually 37°. Following this, the bottom chamber or opposite side of the top chamber may be analysed for cells that have squeezed through the microporous membrane.

[0009] US Patent Specification Nos. 4912057 (Guirguis et al), 5284753 (Goodwin et al), 5302515 (Goodwin et al), 5514555 (Springer et al) and 5601997 (Tchao) are typical examples of these assays. The main disadvantage of the assays described in those specifications

is that the biological process of transmigration through the micropores is difficult to observe due to the geometrical configuration of the apparatus involved. The lens of the optically inverted microscope must be able to focus through the lower chamber and the microporous membrane. This obviously leads to difficulties due to optical aberrations. In effect, the study of the cells morphology changes while transmigrating across the membrane and their subsequent cytoskeletal changes reverting to their former state is a process which is difficult to monitor and record due to limitations with current techniques. In addition, although it is possible to alter such an experiments parameters following the initiation of the experiment, such as the introduction of a second chemoattractant, recombinant or cell-derived, at some specified time after commencing the experiment, it is not possible to distinguish separate effects from each said chemoattractant.

[0010] In addition to cell biology studies, the pharmaceutical industry has major problems in the drug screening process and while high throughput screening (HTS) has been extremely successful in the elimination of the large majority of unsuitable drugs, it has not progressed beyond that and usually, after a successful HTS assay, a pharmaceutical company may still have 7,000 possible drugs requiring assessment. This requires animal trials and anything that can be done to reduce the amount of animal trials is to be desired. Thus, there is a need for new techniques for drug testing in the pharmaceutical industry. The current proposals are to screen the physiological response of cells to biologically active compounds such as described in US Patent Specification No. 6103479 (Taylor). This again, unfortunately, is still a static test. Since the cells are spatially confined with the drug, there may be a reaction but it may not necessarily take place when the cells are free to flow relative to the drug as in, for example, the microcapillaries of the body. There are other disadvantages such as the transport and subsequent reaction of the drug following its injection into the animal. Probably the most important disadvantage is that it does not in any way test, in a real situation, drug efficacy.

[0011] Finally, there are no techniques at the present moment for performing assays to test the interaction of a large number of chosen compounds with living cells while the cells or compounds mimic the in vivo situation of continuous flow.

[0012] While in the description herein, the examples all refer to animal cells and indeed mainly human cells, the invention equally applies to plant cells. The term "sample liquid" refers to a suspension of living cells within a suitable carrier liquid which is effectively a culture medium. More than one cell type may be in suspension. Further, the term "reagent liquid" could be any liquid from a drug under assessment, a poison, a cell nutrient, chemoattractant, a liquid containing other cells in suspension or indeed any liquid who's effect the sample liquid requires assessment.

[0013] The present invention is directed towards providing such methods and apparatus for performing such assays.

Statements of Invention

[0014] The present invention provides a biological assay method comprising:-

delivering a sample liquid of a suspension of cells at a controlled steady flow rate through a biochip in the form of an elongate enclosed microchannel;

causing an externally generated test to be carried out on the sample liquid as it is being delivered through the biochip; and

examining the sample liquid over time to observe the effect of the test on the sample.

[0015] The externally generated test can be carried out in many ways, for example, it can comprise coating the internal bore of the biochip with a protein which could, for example, be an extracellular matrix ligand or could be formed by an endothelium layer which in turn would be formed by seeding the biochip with endothelial cells allowing the cells to grow on the walls. The cells can be taken from an animal or indeed most often from a human, but could also be from a plant. The bore of the biochip, in certain tests, is substantially the same size as the post capillary venules of an animal or, more particularly, a human. With such a method, for example, one can have tests for cell flow, rolling, tethering and migration of previously adhered cells, and adhesion. All of these may be recorded in any suitable manner. It is envisaged that the velocity of the delivery of the sample liquid may be varied to provide different test conditions or the velocity of the delivery of the sample liquid can be increased until previously adhered tests are removed and then the velocity forms a measure of the adherence. Alternatively, a separate flushing liquid may be introduced to remove previously adhered cells, the velocity of the flushing liquid forming a measure of the adherence. Needless to say, after cells have been adhered to the protein, the sample liquid could be replaced by a reagent liquid and the effect of the reagent liquid could be observed. The reagent liquid could be any suitable liquid. One could be, for example, an adhesion detachment reagent liquid and thus the effect of this on the previously adhered cells could be monitored. Needless to say, any reagent liquid may be delivered simultaneously with the sample liquid through the biochip to achieve various tests. For example, it would be possible to deliver a reagent liquid at a controlled steady flow rate through another microchannel connected to the first microchannel, the channels being connected intermediate their ends by an interconnecting channel. The fluid pressure of the liquids could be chosen so as to cause a

diffusion of the reagent through the interconnecting channel or alternatively the fluid pressures could be maintained equal to prevent diffusion of the reagent. Similarly, the channels may be connected intermediate their ends by an interconnecting channel having a restricted entry throat, which restricted entry throat would preferably have a cross sectional area less than that of a cell when the cell is freely suspended in the sample liquid. This would be a very good way of studying the mechanisms involved in cell migration from the endothelium to the extracellular matrix.

[0016] In other embodiments, the bore of the microchannel could be provided with a hydrophobic coating such as liquid silicon.

[0017] It is envisaged that more than one cell type may be held in suspension as this often happens in practice and indeed in many instances, it may be advantageous to deliver a reagent liquid and a sample liquid through the microchannel to form multilaminar flow and then if the reagent liquid comprises a chemoattractant suitable for one of the types of the cell, it will be possible to effectively separate that particular type of cell from the sample.

[0018] Further, the invention envisages a method in which the biochip comprises two microchannels, one a feeding microchannel having a cell reservoir intermediate its ends and the other a reactant microchannel connected to the reservoir by a connecting means comprising:-

storing cells in the cell reservoir;

feeding and growing the cells in the cell reservoir by delivering a culture medium through the feeding microchannel; and

delivering reagent liquid through the reactant microchannel.

[0019] The reagent liquid could, for example, be one or more of a chemoattractant, toxic substance or pharmaceutical preparation and these could be recombinant or cell derived. It is envisaged that a plurality of tests can be carried out simultaneously using the one sample liquid forming portion of a large sample and using different test conditions or alternatively, a plurality of the same tests may be carried out using different sample liquids and the same test conditions.

[0020] According to the invention, there is provided a biochip comprising:-

a pair of enclosed microchannels, namely, a main microchannel and an auxiliary microchannel, each microchannel having a liquid inlet port adjacent its proximal end and at least the main microchannel being an elongate channel and having a liquid output port adjacent its distal end; and

a connection means between the microchannels for transfer of contents therebetween.

Ideally, the outlet port between the feeder microchannel and the main microchannel has a restricted throat. Further, there can be produced a biochip comprising two separate main microchannels and a connecting microchannel connecting the two separate main microchannels. Such separate microchannels can be parallel, diverge towards each other and indeed the connecting channel may also have a restricted throat or the channel itself may just have a restricted cross section.

[0021] Further, there is provided a biochip comprising:-

two separate main microchannels; and

a common microchannel connected to the two main microchannels to provide an extension of the two main microchannels.

[0022] This common microchannel can feed two further microchannels and indeed the microchannel can comprise a main microchannel and a take-off microchannel intermediate its ends, the take-off microchannel having an entrance which projects into the main microchannel to divert flow from the main microchannel into the take-off microchannel. Further, a microwell can be incorporated in a microchannel forming part of a biochip, which microwell may have connected to it a further feeder microchannel delivering into and out of the microwell, the feeder microchannel having an inlet port adjacent its proximal end and an outlet port adjacent is distal end.

[0023] It is envisaged that the microchannel according to the present invention will generally have a planar top wall to allow good optical properties for examination under a microscope and generally speaking, the microchannel comprises planar top, bottom and side walls which side walls taper outwards and upwards away from each other. Ideally, the top wall is removable and is formed from aplastics film.

[0024] Preferably, each port has a bubble release port and valve associated therewith. The cross sectional area of the microchannel is between 25 μm^2 to 10,000 μm^2. and preferably greater than 400 μm^2.

[0025] It is envisaged that assemblies comprising a plurality of biochips as described above will be formed on the one base sheet and will preferably have various common feeder microchannels having ports therein. The advantage of a whole lot of biochips all on the one sheet is that they can be readily easily examined by the one microscope.

[0026] Further, the invention provides a biochip assembly comprising a plurality of separate biochips, each comprising:-

an enclosed elongate microchannel having an inlet port adjacent its proximal end;

an outlet port adjacent its distal end;

a reservoir well for each biochip;

an enclosed main delivery channel feeding a plurality of delivery channels, each having a combined output and input port; and

an enclosed conduit for connecting a delivery channel for interconnection of the ports and for the reservoir wells.

[0027] Ideally, an output reservoir well is associated with each outlet port of each separate biochip and in which an enclosed conduit for the output port of the biochip to its associated output reservoir.

[0028] Ideally, each conduit is releasably connected to each of its associated ports and wells.

[0029] The conduit may ideally be a length of flexible tubing and normally has an internal cross sectional area substantially greater than that of the microchannel.

[0030] In many embodiments, there will be more than two microwells associated with each biochip and indeed, the biochip incorporated therein may be of any of the biochips described above.

Detailed Description of the Invention

[0031] The invention will be more clearly understood from the following description thereof, given by way of example only, with reference to the accompanying drawings, in which-

Fig. 1 is a plan view of a biochip according to the invention,

Fig. 2 is a sectional view along the lines II-II of Fig. 1,

Fig. 3 is a diagrammatic view of an assay assembly according to the invention,

Fig. 4 is a plan view of a biochip assembly utilising the biochips of Fig. 1,

Fig. 5 is a plan view similar to Fig. 1 of an alternative construction of biochip used in the assembly of Fig. 4,

Fig. 6 is an enlarged view of the circled portion identified by the reference letter A in Fig. 1,

Fig. 7 is a further enlarged view of the circled portion identified by the reference letter A in Fig. 6,

Fig. 8 is an enlarged portion of the biochip identified by the letter A in Fig. 1 for a different assay,

Fig. 9 is an enlarged view of the circled portion identified by the reference letter A in Fig. 8,

Fig. 10 is a view similar to Fig. 98 showing a different assay according to the invention,

Fig. 11 is a plan view of a biochip assembly utilising the biochips of Fig. 10,

Fig. 12 is an enlarged view of the circled portion identified by the letter A in Fig. 11 illustrating one assay,

Fig. 13 is a plan view of another biochip according to the invention,

Fig. 14 is an enlarged view of the circled portion identified by the reference letter A in Fig. 13 illustrating another test,

Fig. 15 is a plan view of a still further biochip according to the invention,

Fig. 16 is an enlarged view of the portion identified by the letter A in Fig. 15,

Fig. 17 is a plan view of a still further biochip according to the invention,

Fig. 18 is an enlarged view of the portion identified by the reference letter A in Fig. 17,

Fig. 19 is a plan view of a still further biochip according to the invention,

Fig. 20 is an enlarged view of the portion identified by the reference letter A in Fig. 19,

Fig. 21 is a plan view of a still further biochip according to the invention,

Fig. 22 is an enlarged view of the circled portion identified by the reference letter A in Fig. 21,

Fig. 23 is a plan view of a biochip assembly incorporating the biochip of Fig. 21,

Fig. 24 is an enlarged view of portion of another biochip according to the invention,

Fig. 25 is a plan view of a further biochip according to the invention,

Fig. 26 is an enlarged view of a portion of the biochip identified by the reference letter A in Fig. 25,

Fig. 27 is a perspective view of an alternative construction of biochip assembly according to the invention with portions removed,

Fig. 28 is a sectional view through the biochip assembly, and

Figs. 29 to 32 are plan views of biochip assembly in different positions of use.

[0032] In the drawings, there are described many micro-fabricated biochips having a plurality of ports which it would be very confusing to identify by different reference numerals in the drawings of each microchip or biochip. Thus, all the ports in the drawings are identified by a small number of reference numerals and additional reference letters. However, for clarity in viewing the drawings, this scheme of identification has been adopted even though, at times, the same letter is used to identify different parts

[0033] Referring to Figs. 1 and 2, there is illustrated a biochip, indicated generally by the reference numeral 50 comprising a microchannel 51 formed in a base sheet 52. The microchannel 51 comprises a top wall 53 formed from plastics film and has a planar bottom wall 54 and tapering side walls 55 which taper outwardly away from the bottom wall 54. A fluid inlet port 1 is illustrated in Fig. 2. The biochips 50 are fabricated using standard lithographic and hot embossing techniques. A stainless steel substrate is masked with photoresist (SU-8-5 m, as distributed by Chestech). After ultraviolet lithography, the photoresist mask is delivered and the substrate is electrochemically etched to produce a negative master mould in stainless steel. The remaining mask is subsequently removed. Hot embossing is employed to replicate the microfluidic pattern of the microchannels 51 in a variety of thermoplastic materials such as PMMA, polycarbonate, and polystyrene. The liquid inlet and outlet ports, such as the port 1, are glued in position. The biochip is treated in oxygen plasma (0.1 torr, 80% oxygen and +100V for 30 seconds) to ensure a hydrophilic surface and is subsequently sealed with a pressure-sensitive film (PHARMCAL PM-150-c TC-249 V-232C 150 POLY H9, manufactured by Flexcon). This film is a 1.5 mil top-coated clear polyester film, coated with a permanent adhesive containing a photoluminescent additive, backed with a 1.5 mil polyester release liner. The width of the channels may vary from 20-100 μm and a depth from 20-40 μm. The biochip 50 is thus an optically transparent structure. The biochip 50 illustrated in Fig. 1 has a liquid inlet port 1, a bubble release port 2 incorporating a bubble release valve, a liquid outlet port 3 and a bubble release port 4 associated with the outlet port 3.

[0034] It will be appreciated that to a certain extent, the term "input port" and "output port" is a misnomer since in one circumstance, a port may operate as an input port and in another circumstance, as an output port. The size of the microchannels can vary in cross section from between 5 μm x 5 μm to 100 μm x 100 μm but will generally exceed 20 μm x 20 μm.

[0035] Referring to Fig. 3, there is illustrated an assay assembly, indicated generally by the reference numeral 60, comprising a pump assembly 61 incorporating a syringe pump 62 feeding by means of conduit 63 the biochip 50. An optically inverted microscope 65, connected to a digital camera 66, a recorder 67 and monitor 68 are mounted beneath the biochip 50. An epifluorescence device 69 is also connected to the microscope 65. It will be appreciated that the epifluorescence device 69 may not always be required.

[0036] Referring now to Fig. 4, there is illustrated a biochip, again indicated generally by the reference numeral 50 comprising six individual biochips identified by the subscripts (a) to (f) and described in more detail with reference to Fig. 5 below. In many instances, for ease of reference and to avoid a multiplicity of numerals, 51 usually identifies a microchannel and where there is more than one microchannel, subscript letters are used. The biochips 50(a) to 50(b) have a common inlet port 1 and associated common bubble release port 2.

[0037] This biochip 50 of Fig. 5 is a modified form of the biochip illustrated in Fig. 1 and since it is substantially identical to the biochip in Fig. 1 it is identified by the same reference numeral 50 and similar parts are identified by the same reference numerals. In this embodiment, there is a main microchannel 51(a) and an auxiliary microchannel 51(b). The microchannel 51(b) is a shorter feeder channel and has an inlet port 5 and associated bubble release port 6 connected to the inlet port 5 by a microchannel 51(c). A connection means 51 (c), in this case, a simple tee junction, is provided between the main microchannel 51(a) and the auxiliary microchannel 51(b). The outlet ports 3 and their associated bubble release ports 4 are distinguished by appropriate subscript letters (a) to (f) in Fig. 4, depending on which of the individual biochips 50(a) to 50(f) they are associated with.

[0038] The apparatus described has been used in accordance with the invention to observe and study the flowing and rolling of cells for up to several hours. Various other assays as will be described hereinafter have been carried out, including the transmigration of cells on endothelial ligands or extracellular matrix ligands through a constricted channel. The use of image acquisition and recognition software for observation and data collection, including automatic optical readouts, can be provided and is not described as such apparatus and schemes are well known in the art. The number of biochips forming a biochip is a matter of choice.

[0039] One of the great advantages of the assay assemblythat will become apparent is that a variety of tests can be carried out. However, there is a further advantage in that since these tests occur over relatively long periods of time, of the order of hours or so, it is possible to use the one microscope to carry out a multiplicity of examinations as it is usually only necessary to have the activities recorded at discrete time intervals. Thus, for example, the microscope can be indexed to examine each of the biochips 50(a) to 50(f) by simple manipula-

tion. Further, it will be appreciated that assemblies with greater than six separate biochips mounted thereon, may be advantageous. By using relatively large assay assemblies, that is to say, containing a multiplicity of individual biochips and using the one microscope, it should be possible to carry out a multitude of assays at the same time.

[0040] In the embodiment of Fig. 4, there is a common inlet port 1 and associated bubble release port 2 for the biochips 50(a) to 50(d): it could equally well be separate individual additional inlet ports and associated bubble release ports, as illustrated for the single biochip 50 of Fig. 5.

[0041] The present examples relate mainly to cell assays of humans and the cells are generally contained in the solution of culture medium maintained at 37°C. It is well known that there are some essential nutritional requirements for living human cells and standard culture medium was used. A minimal medium contained glucose as a source of carbon, NH_4Cl as the source of nitrogen and salts such as Na^+, K^+, Mg^+, Ca^+, SO_4^{2-}, Cl^- and PO_4^{3-}. In certain circumstances, in carrying out the tests, when a richer culture medium was required, partly hydrolysed animal or plant tissues rich in amino acids, short peptides and lipids, were used, as well as yeast extract which is rich in vitamins and enzyme cofactors, nucleic acid precursors and amino acids.

[0042] One of the major difficulties in carrying out an assay according to the present invention is to ensure that the flow rate was kept as constant as possible. The problem with variations in flow rates is that they can provide variations in the shear stress on the wall, for example, of a capillary or of a microchannel such as in accordance with the present invention. Typical flow rates in the assays were in the range from 100pl/min to 10 μl/min. The corresponding linear velocities for these flow rates were 0.5 μm/s to 5cm/s respectively.

[0043] In the assays now being described, the microchannels were comparable in size to the post capillary venules in the human bodies and therefore it is suggested that the microchannels imitate the natural environment more closely than any other form of channel. Thus, when dealing with assays concerning venules in the human body, sizes are of the order of 20 μm while for human capillaries, they can be as small as 8μm.

[0044] In the embodiments already described, each port has associated therewith a bubble-release port. This is vital because bubbles in the microchannel structures effectively result in the termination of an assay and therefore cannot be allowed. Initially upon injection into a microchannel of a biochip, the only port that will be open is the bubble-release port. It has been found that the microchannel 51(c) (in Fig. 5) connecting the input or output port to the bubble-release port must be wider than the microchannels in the rest of the structure. The reason for this is that during pressure build-up, the fluid containing bubbles will be released through the wider channel connected to the bubble-release port and not through the microchannels of the remaining structure where the assay will be carried out. Following the release of these bubbles, the bubble-release port is closed and the fluid then flows throughout the microchannel structure. Finally, the length of the microchannel is varied depending on the test being carried out.

[0045] As mentioned already, a pressure-sensitive film is used to cover the biochip effectively sealing the microchannels. Thus, the pressure-sensitive film can be removed after the execution of an assay and accordingly it is possible, prior to removal of the film to inject a solution which fixes cells to the film and the plastic substrate of the biochip enabling further study. The pressure-sensitive film may obviously be removed and the cells taken away for additional research.

[0046] Referring to Figs. 1 and 2, to study the flow, rolling and migration of cells, a ligand, namely, an adhesion mediating protein, is injected into the port 2 and the inner bore of the microchannel 51 is coated with the protein. This is then stored to allow adherence of this ligand to the walls 53, 54 and 55 of the microchannel 51. In this embodiment, the microchannel was approximately 20 cm in length. A liquid sample carrying a specific cell in suspension was then injected into the port 2 and the subsequent progress of the cells was observed.

[0047] In the various embodiments, the cells are identified by the reference letter C and by suitable lowercase lettering in brackets. Similarly, the arrow F indicates the direction of flow of the liquid sample and the letter L identifies the ligand. Cells C(a) can be observed as flowing normally through the microchannel 51 while finally the cell C(c) is starting to adhere to the ligand L. This observation takes place at the circled portion A of Fig. 1 or Fig. 5 and is illustrated in Figs. 6 and 7.

[0048] Referring now to Fig. 7, the cell C(c) is shown just beginning to attach to the ligand L. The cell C(d) is shown adhering strongly to the ligand L, in this case, the protein, on the channel wall 54 with tethers or adhesion plaques, identified by the reference C_1. Finally, the cell C(e) is shown starting to migrate away from the ligand Lwith the leading edge of the cell C(e) starting to pull away from the ligand L with a leading tether C_1 starting also to elongate and break its contact with the ligand L.

[0049] Referring now to Figs. 8 and 9, in this assay, the ligand was provided by the seeding and subsequent growth of endothelial cells. This ligand is shown and identified by the letter L and the cells are identified by the same reference numerals. Strictly speaking, the ligands which are available to bind to the receptors on the cells C(c) are on the surface of the endothelium cells. Endothelial cells were chosen as a HUVEC cell line.

[0050] Referring again to Fig. 4, it will be seen that the biochip 50 consists of six biochips 50, all of which can be used for the test previously described. It will be appreciated that, for example, many other tests can be carried out simultaneously. For example, the common port 1 can be used, for example, to coat all the biochips 50 (a) to 50(f) with the one ligand or to inject the one sample

liquid.

[0051] Therefore, variations of the test can be carried out such as, for example, assaying one cell type and several ECM ligands. Then each of the biochips 50 would be coated with a different adhesion mediating ligand. Using the same pumping system, you inject at port 5(a) with flow through the output port 3(a) for 50(a), you inject at port 5(b) and the output at port 3(b) for 50(b), and so on. Having coated all the microchannels with the chosen ECM ligands, the specified cell type is injected then through the common port1. This allows the researcher to build up a profile of the characteristic behaviour of a cell type in response to particular ECM ligands. The same test can then be carried out using different cell types and one ECM ligand in which case the ECM ligand would be injected at port 1 with outputs at ports 3(a) to 3(f) followed by subsequent injection of different cell types into each of the biochips 50(a) to 50(f) injecting at port 5(a) to 5(f). This will allow the option of classifying an ECM ligand according to the behaviour of different cell types with regard to the multistep progress of rolling, tethering, adhesion and subsequent migration. Similarly, this can be done for several cell types with the one endothelial layer.

[0052] Still dealing with the apparatus and the biochips described above , it is possible to carry out a cell binding assay to identify proteins which will cause specific adherences of particular cell types. From the known initial concentration of cells passed through the biochip during the course of the assay, it is possible to obtain an accurate statistical and qualitative result regarding the percentage of cells which adhered to the coated walls, providing a clear quantitative result for the adhesion affinity of a specific ECM ligand. Here the adhesion affinity refers to the response of cell by adhesion to the ECM ligand-coated channel; i.e. the greater the number of cells adhered to a particular ECM ligand, the greater the adhesion affinity of that ligand. In addition, knowing the velocity of cells within the channels and the length of the channels themselves, it is also possible to obtain a clear physical result regarding the response time of the cell type to its environment. Thus, it is possible to calculate how long it takes the cell to react to its surroundings based on its site of adhesion within the microchannel structure, for example, a cell type has attached to the chosen ECM ligand or ligands, coating the microchannel walls image acquisition and recognition software may be employed to execute an automated based image acquisition or recognition of the cell type or indeed carry out any form of manual cell count.

[0053] Thus, for example, it is possible to do any of the following tests-

- One cell type and one ECM ligand
- One cell type and endothelium layer ligand
- One cell type and several ECM ligands
- Several cell types and one ECM ligand
- Several cell types and endothelium layer ligand

[0054] Obviously, various other variations, for example, various cell types and many ligands may also be used. The permutations and combinations are endless.

[0055] Finally, the binding affinity can be calculated from the shear stress required to cause dissociation of bound cells. By increasing the flow of velocity in the microchannel until there is dissociation of cells from the walls, it is possible to get a measure of the relative binding strengths of various ligands. Therefore, from the strength of the shear stress or corresponding velocity causing dissociation, this can be related to the binding affinity which a particular cell type has for a corresponding adhesion-inducing and mediating ligand. Needless to say, this could be applied to all the assays that have been carried out already. Any flushing liquid may be used, even the sample liquid itself.

[0056] Referring now to Fig. 9, there is illustrated another assay in a view similar to Fig. 7 in which parts similar to those described with reference to the previous drawings are identified by the same reference numerals. In this assay, following adhesion of the cell type to the corresponding adhesion-inducing and mediating ECM ligand, an adhesion inhibiting reagent, recombinant or cell derived, is injected at port 1 with an output at port 3, of the biochip 50 of Fig. 1. The cell C(f) can be seen securely anchored to the ligand L, then as C(g) beginning to separate and finally at C(h) having separated totally from the ligand. After the dissociation of the cell type from the chosen ECM ligand coating the microchannel walls, it is possible to use image acquisition/recognition software to do an automated based, image acquisition/recognition of cell type or manual cell count to calculate how many cells have responded by clear dissociation from the adhesion-inducing/mediating ECM ligand(s), again providing a clear result for the dissociation affinity of a specific reagent. Here the dissociation affinity refers to the response of a cell by dissociation from the ECM ligand-coated channel; i.e. the greater the number of cells dissociated from the particular ECM ligand, the greater the dissociation affinity of that reagent. Since the percentage of cells from the initial sample of known cell concentration is known, the dissociation affinity results in determination of the percentage of the adhered cells which subsequently dissociated. An identical test can be done for an endothelium layer and one detachment reagent. Then, using the assay assembly 60, many variations on the test can be carried out which will be easily apparent, whether they be one cell type and several ECM ligands and one or more detachment reagents; one ECM ligand, several cell types and one or more detachment reagents; several cell types, one endothelium layer and one or more detachment reagents. Obviously, all these variations will be readily apparent once it is appreciated that the assay assembly 60 is available.

[0057] Referring to Fig. 11, the biochip, again identified by the reference numeral 50 and various subscript letters, is substantially similar to the biochip 50 of Fig.

4, except that there is a further auxiliary microchannel with an outlet port 7 and associated bubble port 8, again differentiated by appropriate letters (a) to (f).

[0058] The construction of biochip and biochip assembly of Figs. 4, 5 and 11 is particularly useful for chemokine, cytokine and chemoattractant induced cellular arrest assays.

[0059] Referring again, for example, to Fig. 5, the biochip 50 can be used in assays to determine whether a cell, for example, a lymphocyte crawling in response to a particular chemoattractant is integrated with the endothelial or ECM ligand utilised. Again, in this assay the walls of the microchannel are coated with a single adhesion-mediating/inducing ECM injected at port 1, and outputted at port 6. Once it is ready, then essentially the same assay as carried out before with a subsequent measurement of the crawling caused by the chemoattractant which is injected at port 4 with an output at port 6. Fig. 12 which is substantially similar to Fig. 6 illustrates this. Needless to say, instead of using an ECM ligand, an endothelium layer may also be used as illustrated in Fig. 10.

[0060] Then, to investigate cellular navigation, the biochip assembly of Fig. 11 may be used to determine whether a cell crawling in response to chemokine is integrated with the nature of the ECM ligand used. Thus, for example, one ECM protein may be injected at port1 with outputs at ports 3(a), 8(a), 5(a)for biochip 50(a), and so on. Then, a particular cell type can be injected at port 3(a) with output at port 5(a) for biochip 50(a), port 3(b) for biochip 50(b) and so on. A chemoattractant would then be injected in port 8(a) with output at port 5 (a) for biochip 50(a) and then a second different chemoattractant would be injected at port 8(b) with an output at port 5(b) for the biochip 50(b) and so on. Additionally, the assay can be used using multiple cell types, one ECM ligand and several chemoattractants, and so on. In other words, there are many permutations and combinations to study cellular navigation.

[0061] Cellular activation can be studied using the biochip 50 of Fig. 11. The purpose of the assay is to determine if the nature of the cell (e.g. lymphocyte) activation determines binding specificity or preference for either the ECM ligand or an individual chemoattractant migratory signals. In this case, the microchannels of each individual biochip 50(a) to 50(f) are individually coated with specific matrix ligands, e.g. fibronectin, collagen or hyalaronic acid in the case of lymphocytes injected at port 3(a) with output at port 5(a) for biochip 50(a), injected at port 3(b) and output at port 5(b) for biochip 50(b), and so on. Cells are permitted to crawl through a protein coated channel before encountering multiple channels coated with individual matrix molecules by injecting the protein at port 1, output at ports 3(a) to 3(f), injecting the cell type at port 1 with output at ports 7(a) to 7(f). The choice of channel can be analysed with response to the nature of the cell activation or nature of the chemoattractant signalling. In this case, one or several chemoattractants may be incorporated, for example, injecting the first chemoattractant at port 5(a) with output at port 3(a) 6 for biochip 50(a), injecting the next chemoattractant at port 5(b), output at port 3(b) for biochip 50(b), and so on. Hence, cells will be activated utilising multiple individual signals. The effects of such activations can be studied to determine whether the nature of activation determines either ligand or chemoattractant preference.

[0062] It will be appreciated that these tests can be carried out using separate cell types, several ECM ligands and several chemoattractants. Again, the variations are endless Indeed, one cell type, an endothelium layer and several chemoattractants may be assayed, as can several cell types, the one endothelium layer and several chemoattractants.

[0063] Referring to Figs. 13 and 14, there is illustrated an alternative construction of composite symmetrical biochip, again identified by the reference numeral 50 in which there are two microchannels, namely a main microchannel 51(a) and an auxiliary microchannel 51(b) joined by connecting means 51(c) in the form of a connecting microchannel 51(c). In the microchannel 51(b), chemoattractant can be injected through the port 7 and the sample liquid through the port 1 in the microchannel 51(a). Then, by judicious choice of the fluid flow of both the chemoattractant and the sample liquid, it is possible to introduce flow between the two channels through the connecting microchannel 51(c), as illustrated in Fig. 14. Alternatively, it is possible to fabricate, effectively what is now a symmetrical biochip, in such a way that the pressure of the flow of the chemoattractant is equal to the pressure of the flow of the sample thereby resulting in no diffusion or leakage at the interconnecting channel with any movement of the cell through the connecting channel being caused by the chemoattractant.

[0064] Figs. 15 and 16 and Figs. 17 and 18 show still further alternative embodiments with views similar to Figs. 13 and 14 respectively, except that the main microchannel 51(a) is illustrated at the top of the drawing.

[0065] Referring to Figs. 19 and 20, there is illustrated an alternative construction of biochip, again indicated generally by the reference numeral 50. In this embodiment, there is provided an additional inlet port 5 as heretofore, however, in this embodiment, the connection between the inlet port 5 and the main microchannel 51(a) is through an auxiliary feeder microchannel 51(b) having a restricted throat 51(r), as shown in Fig. 20. Essentially, this is for the injection of a chemoattractant such that the entrance diameter, that is to say, the cross-sectional area of the throat 51(r) is smaller than the cross-sectional area of the cell C under examination when the cell C is in free suspension and flowing as illustrated in Fig. 20. Thus, for example, a cell C(a), on approaching the throat 51(r), has to squeeze itself through as illustrated by the cell C(b) for exiting the throat. Figs. 19 and 20 show one cell type, one ECM ligand and one chemoattractant. Needless to say, using the same arrangement of biochip, as illustrated in Fig. 11, with the restricted

throat 56, multiple assays may be carried out. Accordingly, for example, assays with one cell type, one ECM ligand and one chemoattractant, or with one cell type, one endothelium layer and one chemoattractant, may be carried out with one biochip 50. However, for one cell type, one ECM ligand and several chemoattractants or several cell types, one ECM ligand and several chemoattractants, or one cell type, several ECM ligands, several chemoattractants, or several cell types, several ECM ligands and several chemoattractants, or one cell type, one endothelium layer and several chemoattractants, or indeed, several cell types, endothelium layer and several chemoattractants may all be assayed with the biochip.

[0066] Similarly, the same arrangements of Figs.13 and 14; Figs. 15 and 16 and Figs. 17 and 18 could all be used subject to a restricted throat being provided. Needless to say, all of these embodiments may be altered in order to encompass several interconnecting sections enabling the execution of several separate tests in parallel.

[0067] Referring to Figs. 21 and 22, there is provided a biochip again indicated generally by the reference numeral 50, comprising two microchannels 51(a) and 51 (b) connected by a connecting means 51(c), namely a common microchannel 51(c). The main microchannels 51(a) has an input port 1 and an output port 3 and the other auxiliary microchannel 51(b) has an input port 5 and output port 7. All these ports have associated bubble-release ports 2, 4, 6, and 8 respectively. Parts similar to those described with reference to the previous drawings are identified by the same reference numerals. When it is desired to assay chemotaxis, namely, active swimming, towards chemoattractants, whether such chemoattractants are recombinant or cell-derived, the inlet port 1 has a chemoattractant CA delivered therethrough and the inlet port 5 has the sample liquid. These are identified as two streams, the chemoattractant CA and the liquid sample again carrying cells C in suspension. Fig. 22 shows that as the liquid sample progresses, some of the cells, identified by C(a), progress into the chemoattractant CA . The connecting microchannel 51 (c) has multi-laminar flow of the chemoattractant CA and the liquid sample carrying the cells C. If the channel length L is shorter than the diffusion of the chemoattractant or culture medium, then the assay will determine whether a cell's receptors may be activated prior to adhesion to a substrate.

[0068] Ideally, the microchannels of the biochip 50 are coated with a liquid silicone to provide a hydrophobic surface and thus the cells will not adhere to the microchannel walls so that any movement towards the chemoattractant will be solely due to active swimming and not to adhesions followed by subsequent migration.

[0069] Referring now to Fig. 23, there is illustrated a biochip again identified generally by the reference numeral 50, incorporating the biochips of Fig. 21, identified again by the reference numeral 50 and the letters (a) to (f). In this particular biochip 50, there is a common port 37 which connects to all of the interconnecting channels and thus several cell types and one chemoattractant may be tested or several chemoattractant and the one cell. For example, the biochip of Fig. 23 could be used to filter out one cell type for collection from another. Therefore, they could be in the form of a cell filtering or separation of one or more cell types from samples.

[0070] Referring to Fig. 24, there is illustrated portion of a biochip, again identified by the reference numeral 50 comprising two portions of a main microchannel 51 (a), identified as 51(a)' and 51(a)", and two portions of an auxiliary microchannel 51(b), identified as 51(b)' and 51(b)", connecting into a common connecting microchannel 51(c). The sample liquid contains three cell types, namely, C(a), C(b) and C(c). In use, chemoattractant CA, the flow being identified by the arrows and the chemoattractant by cross hatching, having an affinity with cells C(a) is fed through the feeder or auxiliary microchannel 51(b)' to establish multilaminar flow with the sample liquid fed from the microchannel 51(a)'. The cells C(a) migrate into the chemoattractant CA and out the take-off microchannel 51(b)" formed by the next portion of the auxiliary microchannel 51(b) with the chemoattractant CA. The cells C(b) and C(c), due to the laminar flow in the connecting microchannel 51(c), progress out through the microchannel 51(a)'. This can be repeated as often as necessary. Further, many ways of take-off of the chemoattractant may be provided.

[0071] Needless to say, as in previous embodiments, it is possible to incorporate several interconnecting channels which allows the analysis of several different cell types interacting with several different drugs or chemoattractants. Also, more than two microchannels may be used and interconnected, either all together or in combinations, e.g. pairs. Also, following the analysis of the interaction between the drug or chemoattractant and the cell type, it is possible to coat the microchannel walls with individual specific ECM ligands, and so on. In other words, the variations of the tests already described may also be carried out with this arrangement.

[0072] Referring now to Figs. 25 and 26, there is illustrated another construction of biochip again indicated generally by the reference numeral 50, in which parts similar to those described with reference to the previous drawings are identified by the same reference numerals. There is a main microchannel 51(a) which has an inlet port 2 and an outlet port 3. There is connected a microwell 58 intermediate the ends of the main microchannel 51(a). A further auxiliary microchannel 51(b) having an inlet port 5 adjacent its proximal end and an outlet port 7 adjacent its distal end, feeds the microwell 55. The top of the microwell 58 thus forms the connecting means between the two microchannels.

[0073] In use, a sample liquid, again a culture medium containing cells C, is stored in the microwell 58 and a suitable culture medium food is delivered through the inlet port 3 through the microchannel 51(b) and then out

from the microwell 55 through the outlet port 7. This culture medium food is constantly flowing through the microwell 55 in order to feed the cells. In the upper main microchannel 51(a), a drug and/or chemoattractant and/or toxic sample is delivered through the inlet port 2 through the microchannel 51(a) and out the outlet port 6. The pressure of the fluid flow in themain microchannel 51(a) is slightly greater than in the auxiliary microchannel 51(b) and thus there is a slow diffusion of the drug or the chemoattractant or the toxic sample into the microwell 50.

[0074] This assay enables the study of the behaviour of the cells, for example, migration towards or away from the particular drug or chemoattractant or possibly the detrimental or even stimulating effects on the cells due to the introduction of the proposed toxic sample or reagent. This would be a very good way for testing the toxicity of drugs. Similarly, if a chemoattractant were introduced during the culture and growth of the cell type, it would determine the effect, if any, on its growth. Again, it is possible to coat the microchannel walls and the microwell walls with individual specific ECM ligands or endothelium layer. Again, several interconnecting channels may be coated with different ECM ligands facilitating the contemporaneous analysis of one or more cell types or chemoattractant drugs. Again, it will be appreciated that the biochip 50 could form part of a larger assay assembly. This also shows that the function of main and auxiliary microchannels may be reversed.

[0075] Referring to Figs. 27 to 32 inclusive, there is illustrated a further biochip. While, strictly speaking, it should be called a biochip in this description, to distinguish it somewhat from the other embodiments, it is hereinafter described as a biochip assembly because of the fact that it incorporates certain additional features over and above the biochips previously described. The biochip assembly is illustrated and indicated generally by the reference numeral 100 and like the other biochips previously described, is manufactured by a clear plastics material, however, certain portions, which can be partially seen in Fig. 27, are illustrated. The biochip assembly comprises a plurality of biochips, indicated generally by the reference numeral 50, each having an input port 1 and an output port 3 Individual biochips are not identified by subscript letters as it would only confuse the description. The biochips 50 are manufactured in substantially the same way as the previously described biochips, as are the input ports 1 and output ports 3. There is provided a pair of reservoir wells 101 adjacent each inlet port 1 and two output reservoir wells 102 adjacent each outlet port 3. There is further provided a main delivery channel 103 having an inlet port 104 which in turn feeds through further delivery channels 105 each having a combined input/output port 106. An enclosed conduit 107, in this case provided by a length of plastics tubing, interconnects the various ports and/or the wells, as will be described hereinafter. The internal cross-sectional area of the conduit 107 is considerably greater than that of the microchannel.

[0076] Above the reservoir wells 101 and 102, there is provided conduit supports comprising a bridge plate 108 having holes 109 which is mounted above and spaced-apart from the respective reservoir wells 101 and 102, only one bridge plate 108 is illustrated in Fig. 27.

[0077] In operation, for example, when conducting a cell adhesion study, a syringe pump is connected to the biochip assembly 100 through the inlet port 104. For example, different ligands could be provided in each reservoir well 101 and the same cell sample in each of the other of the pair of reservoir wells 101. These could be pipetted in or provided in any known way. Then the conduit 107 is connected between initially the well 101 containing the ligand. The ligand is then drawn into the conduit 107, as illustrated in Fig. 29. Then, the conduit 107 is connected to each of the input ports 1 and the ligand delivered through each biochip 50 to coat the interior of the microchannel. After coating, a suitable culture medium solution can be delivered through the main delivery channel 103 and through the combined input/output ports 106 through the conduit 107 and then through the microchips 50 (Fig. 30). Then, the conduits 107 which form sample holders can be disposed of and replaced with new sample holder conduits 107 which would be each dipped into the other of the reservoir wells 101 where the cell suspension will then be aspirated into the conduit 107, as shown in Fig. 31. Then, as shown in Fig. 32, the conduit 107 would be again connected to the input port 1 and a further conduit 107 would be connected to each output port 3 and to one of the output reservoir wells 102, as illustrated in Fig. 32. The assay is then carried out.

[0078] It will be appreciated that it would be possible to use a plurality of biochips in series. Thus, for example, rather than one array of biochips, as illustrated, there could be further arrays of biochips. Further, by having the output reservoir wells 102, it is possible to conduct further post-analysis work on the samples. It will be appreciated therefore that the biochip assembly 100 essentially comprises four sections, namely, a flow splitter section, sample preparation section, analysis and a post-analysis section.

[0079] It will be appreciated that the conduits are essentially disposable sample holders. It will also be appreciated that in most cases, biological assays are a multi-stage process and thus requires consecutive injection of several samples into the one microchannel. Thus, an ability to dispose of the sample holder tube or conduit contaminated with one sample and replace it with a new uncontaminated tube, is particularly important. It is also important to avoid the contamination of any of the other parts of the biochip and thus cross contamination.

[0080] It will be appreciated that the biochips incorporated can be any of the biochips as previously described.

[0081] It will also be appreciated that it is advantageous to be able to collect the samples from the output of the analysis section, that is, where the biochips 50 are situated. In many situations, for example, gene expression of sample cells which did not react with a particular ligand may be required. Similarly, waste ligand solution can be stored in one of the output reservoir wells. It will also be appreciated that additional reservoir wells may be provided and that further, additional sets of biochips may also be provided.

[0082] Ideally, the biochip assembly 100 will be manufactured in substantially the same way as the biochips previously described.

[0083] One of the great advantages of using the biochips in accordance with the present invention is the reduction in reagent or sample consumption. It will also allow reduced analysis times and larger transfer rates due to the diminished distances involved. Additionally, in running several assays in parallel, each process in an assay can be manipulated step by step through computer control enabling great efficiency. Again, this accuracy in combination with higher yields, leads to a reduction in waste. This is not only more economically favourable but also environmentally beneficial where hazardous chemicals are involved.

[0084] In addition to chemical production, there are numerous other fields in which the micro devices according to the present invention can make a contribution, such as microbiology, pharmacy, medicine, biotechnology and environmental and materials science. The present invention is particularly adapted to the field of drug discovery and combinatorial chemistry. Again, there should be considerable cost savings for pharmaceutical companies. One of the great advantages of the present invention is that it mimics in vivo testing. Obviously, with the present invention, there is a constant flow of cells and the drug candidate, together with the micro capillary under observation, produces much more accurate statistical results.

[0085] One of the problems with current toxicity tests is that the systems implemented are not always representative of those in vivo providing results which are not characteristic of the in vivo situation. Secondly, there are differences with culturing and maintaining certain cells in vitro. The present invention allows one to simulate in vivo conditions eliminating many of the disadvantages of the present testing and hence immediately decreasing the necessity for animal trials while simultaneously increasing the statistical response as a result of the continuous flow assay according to the present invention.

[0086] One of the major problems with all drug testing is that clinical trials involve testing of the new drug in humans and because of the rigorous testing involved in a new drug, the time and cost of bringing a drug to market is enormous. It is for this reason that pharmaceutical companies must be extremely accurate with results obtained through experimental assays before presenting a new drug for clinical trials.

[0087] One of the advantages of the present invention is that relatively small volumes of blood can be used for analysis in hospitals which can be extremely advantageous. A particular advantage of the present invention is that the biochips are disposable.

[0088] The present invention essentially provides techniques for performing assays that test the interaction of a large number of chosen compounds, for example, candidate drugs or suspected toxic samples with living cells while the cells and/or the compounds mimic the in vivo situation of continuous flow. The assays according to the present invention imitate as far as possible the natural situation, while additionally overcoming the disadvantages of other techniques resulting in a fast and accurate process.

[0089] It will be appreciated that since the biochips are fabricated from a plastics material, it is considerably less expensive than, for example, silicone micro-machining which is often used at present, for such microchips.

[0090] One of the great advantages of plastics material is that it enables real-time monitoring with relative ease, by use of a inverted microscope.

[0091] The size of the microchannels is also significant. Dimensions below the order of 1 mm have long be avoided due to the many difficulties that occurred when scaling down. Such difficulties involve the control of flow within these microchannels.

[0092] While in the present invention, many tests have been tried and described, it will be appreciated that many other assays and tests can be carried out in accordance with the present invention. Indeed, some of the tests according to the present invention are not so much tests, as indeed filtering operations.

[0093] In the specification the terms "comprise, comprises, comprised and comprising" or any variation thereof and the terms "include, includes, included and including" or any variation thereof are considered to be totally interchangeable and they should all be afforded the widest possible interpretation.

[0094] The invention is not limited to the embodiments hereinbefore described but may be varied in both construction and detail.

Claims

1. A biological assay method comprising:

   delivering a sample liquid of a suspension of cells at a controlled steady flow rate through a biochip in the form of an elongate enclosed microchannel;

   causing an externally generated test to be carried out on the sample liquid as it is being delivered through the biochip; and

   examining the sample liquid over time to ob-

serve the effect of the test on the sample.

2. A method as claimed in claim 1 comprising coating the internal bore of the biochip with a ligand formed by a protein.

3. A method as claimed in claim 2 in which the ligand is an adhesion inducing extracellular matrix.

4. A method as claimed in claim 2 or 3 in which the ligand is an endothelium layer formed by:

seeding the biochip with endothelial cells; and

allowing the cells to grow on the interior of the bore of the microchannel.

5. A method as claimed in any preceding claim in which the cell is taken from an animal and the bore of the biochip is chosen to be substantially the same size as the post capillary venules of the animal.

6. A method as claimed in any preceding claim in which one or more of cell flow; rolling; tethering and migration of previously adhered cells; and adhesion is recorded.

7. A method as claimed in any preceding claim in which the velocity of the delivery of the sample is varied to provide different test conditions.

8. A method as claimed in claim 7 in which the velocity is increased until previously adhered cells are removed the velocity forming a measure of the adherence.

9. A method as claimed in claim 7, in which a new flushing liquid is introduced to remove the previously adhered cells, the velocity of the flushing liquid forming a measure of the adherence.

10. A method as claimed in any preceding claim in which after the cells have adhered to the ligand the sample liquid is replaced by a reagent liquid and the effect of the reagent liquid observed.

11. A method as claimed in claim 10 in which the reagent liquid is an adhesion detachment reagent.

12. A method as claimed in any preceding claim in which a reagent liquid is delivered simultaneously with the sample liquid through the biochip.

13. A method as claimed in any preceding claim comprising delivering a reagent liquid at a controlled steady flow rate through another microchannel connected to the microchannel for the sample liquid by an inter-connecting channel intermediate their ends.

14. A method as claimed in claim 13 in which the fluid pressure of the liquids is so chosen as to provide a diffusion of the reagent through the interconnecting channel and into the sample liquid.

15. A method as claimed in claim 14 in which the fluid pressure of the liquids is the same.

16. A method as claimed in any of claims 13 to 15 in which the interconnecting channel is so chosen as to have a constricted entry port between it and the microchannel carrying the sample liquid.

17. A method as claimed in claim 16 in which the constricted entry port is so chosen that its cross-section is less than that of a cell freely suspended in a sample liquid.

18. A method as claimed in any of claims 13 to 17 comprising coating the bore of the microchannel with a hydrophobic coating.

19. A method as claimed in claim 18 in which the hydrophobic coating is a liquid silicon.

20. A method as claimed in any preceding claim in which the sample liquid contains more than one cell type in suspension.

21. A method as claimed in any of claims 1 to 6 comprising delivering a reagent liquid and the sample liquid through the microchannel to form multilaminar flow.

22. A method as claimed in claim 21 in which the sample liquid comprises more than one type of cell in suspension and the reagent is a chemoattractant suitable for one type of cell so as to remove the cells of that cell type from the sample liquid.

23. A method as claimed in claim 22, in which the cells are removed by direction into another microchannel.

24. A method as claimed in any of claims 1 to 15 in which the there are two biochips one a feeding biochip having a cell reservoir intermediate its ends and the other a reactant biochip connected to the reservoir by a channel the method comprising:

storing cells in the cell reservoir feeding and growing the cells in the cell reservoir by delivering a culture medium through the microarray of the feeding biochip; and

delivering reagent liquid through the microarray

of the reactant biochip.

25. A method as claimed in any preceding claim in which the reagent liquid is recombinant.

26. A method as claimed in any preceding claim in which the reagent liquid is cell derived.

27. A method as claimed in any preceding claim in which the reagent is one of a chemoattractant, toxic substance or pharmaceutical preparation.

28. A method as claimed in any preceding claim in which a plurality of tests are carried out simultaneously using a sample liquid forming portion of a larger sample and using different test conditions.

29. A method as claimed in any preceding claim in which a plurality of tests are carried out simultaneously using different sample liquids and the same test conditions.

30. A biochip (50) comprising:-

a pair of enclosed microchannels, namely, a main microchannel (51(a)) and an auxiliary microchannel (51(b)), each microchannel (51(a), 51(b)) having a liquid inlet port (1, 5) adjacent its proximal end and at least the main microchannel (51(a)) being an elongate channel and having a liquid output port (3) adjacent its distal end; and

a connection means (51(c)) between the microchannels (51(a)), 51(b)) for transfer of contents therebetween.

31. A biochip (50) as claimed in claim 30, in which the connection means (51(c)) is an additional inlet port in the main microchannel (51(a)), the port having a restricted throat (51(r)).

32. A biochip (5) as claimed in claim 30, in which the auxiliary microchannel (51(b)) has an outlet port (5) and the connection means (51(c)) comprises a separate connecting microchannel intermediate the inlet ports (1, 3) and outlet ports (5, 7) of each microchannel (51(a), 51(b)).

33. A biochip (50) as claimed in claim 32, in which the connecting microchannel has an internal bore less than that of the main microchannel.

34. A biochip as claimed in claim 32 or 33, in which the main microchannel 51(a) and the auxiliary microchannel 51(b) are parallel on either side of the connecting microchannel.

35. A biochip as claimed in claim 32 or 33, in which the microchannels (51(a), 51(b)) converge towards and then diverge away from the connecting microchannel.

36. A biochip as claimed in claim 30, in which the auxiliary microchannel (51(b)) has an outlet port (7) and the connection means (51(c)) comprises a common port in both microchannels intermediate their inlet ports (1, 5) and outlet ports (3, 7).

37. A biochip (5) as claimed in any of claims 32 to 36, in which the connection means (51(c)) incorporates a restricted throat (51(r)) adjacent the main microchannel (51(a)).

38. A biochip (50) as claimed in claim 30, in which the connection means (51(c)) comprises a common portion of each microchannel (51(a), 51(b)) intermediate their proximal and distal ends.

39. A biochip (50) as claimed in claim 38 in which the bore of the common microchannel has a cross-sectional area the aggregate of the cross-sectional area of the pair of microchannels.

40. A biochip (50) as claimed in any preceding claim, in which the main microchannel (51(a)) incorporates a microwell (58) communicating with the connection means (51(c)).

41. A biochip (50) as claimed in any of claims 30 to 40 in which the microchannel (51) comprises a planar top wall (53).

42. A biochip (50) as claimed in any of claims 30 to 41 in which the microchannel comprises a planar top wall (53), bottom wall (54) and side walls(55).

43. A biochip (50) as claimed in claim 42, in which the side walls (55) taper outwardly and upwardly away from each otherfrom the bottom wall (54).

44. A biochip (50) as claimed in any of claims 41 to 43, in which the top wall (53) is removable.

45. A biochip (50) as claimed in any preceding claim in which the microchannel side walls (55) and bottom walls (54) are formed in a planar sheet of plastics material and the top wall (53) is formed by a plastics film adhered to the sheet.

46. A biochip (50) as claimed in any of claims 30 to 45 in which each port (1, 3, 5, 7) has a bubble release port (2, 4, 6, 8) and a valve associated therewith.

47. A biochip (50) as claimed in any of claims 30 to 46 in which the cross-sectional area of the cell is be-

tween 25 μm^2 to 10,000 μm^2.

48. A biochip (50) as claimed in any of claims 30 to 46 in which the cross sectional area of the cell is in excess of 400 μm^2.

49. A biochip assembly comprising a plurality of biochips (50) as claimed in any of claims 30 to 48 formed on the one base sheet.

50. A biochip assembly as claimed in claim 49 in which a common feeder microchannel having a port therein is connected to each of the biochips.

51. A biochip assembly (100) comprising a plurality of separate biochips (50), each comprising:-

an enclosed elongate microchannel having an inlet port (1) adjacent its proximal end;

an outlet port (3) adjacent its distal end;

a reservoir well (101) for each biochip;

an enclosed main delivery channel (103) feeding a plurality of delivery channels (105), each having a combined output and input port (106); and

an enclosed sample holder conduit (107) for connecting a delivery channel (105) for interconnection of the ports (106, 1, 3) and for the reservoir wells (101, 102).

52. A biochip assembly (100) as claimed in claim 51 in which an output reservoir well (102) is associated with each outlet port (3) of each separate biochip (50) and in which an enclosed conduit (107) for the output port (30) of the biochip (50) to its associated output reservoir (101) is provided.

53. A biochip assembly (100) as claimed in claim 51 or 52 in which each conduit (107) is releasably connected to each of its associated ports (106, 1, 3) and wells (101, 102).

54. A biochip assembly (100) as claimed in any of claims 51 to 53 in which the conduit (107) is a length of flexible tubing.

55. A biochip assembly (100) as claimed in any of claims 51 to 54 in which the conduit (107) has an internal cross-sectional area substantially greater than that of the microchannel.

56. A biochip assembly (100) as claimed in any of claims 51 to 55, in which there is more than two microwells (101, 102) associated with each biochip.

57. A biochip assembly as claimed in any of claims 51 to 56, in which each separate biochip (50) is a biochip as claimed in any of claims 30 to 48.

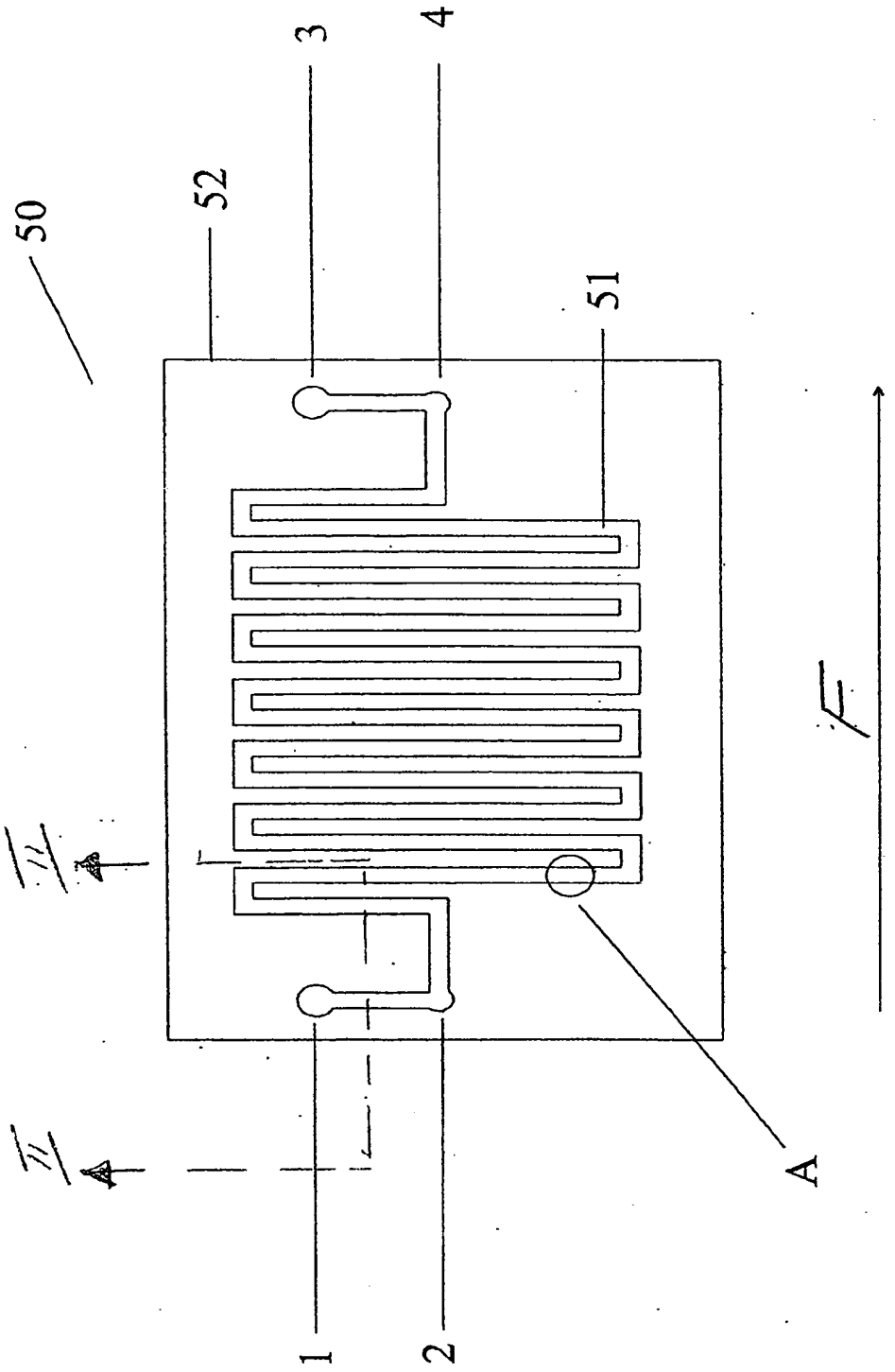


Fig. 1

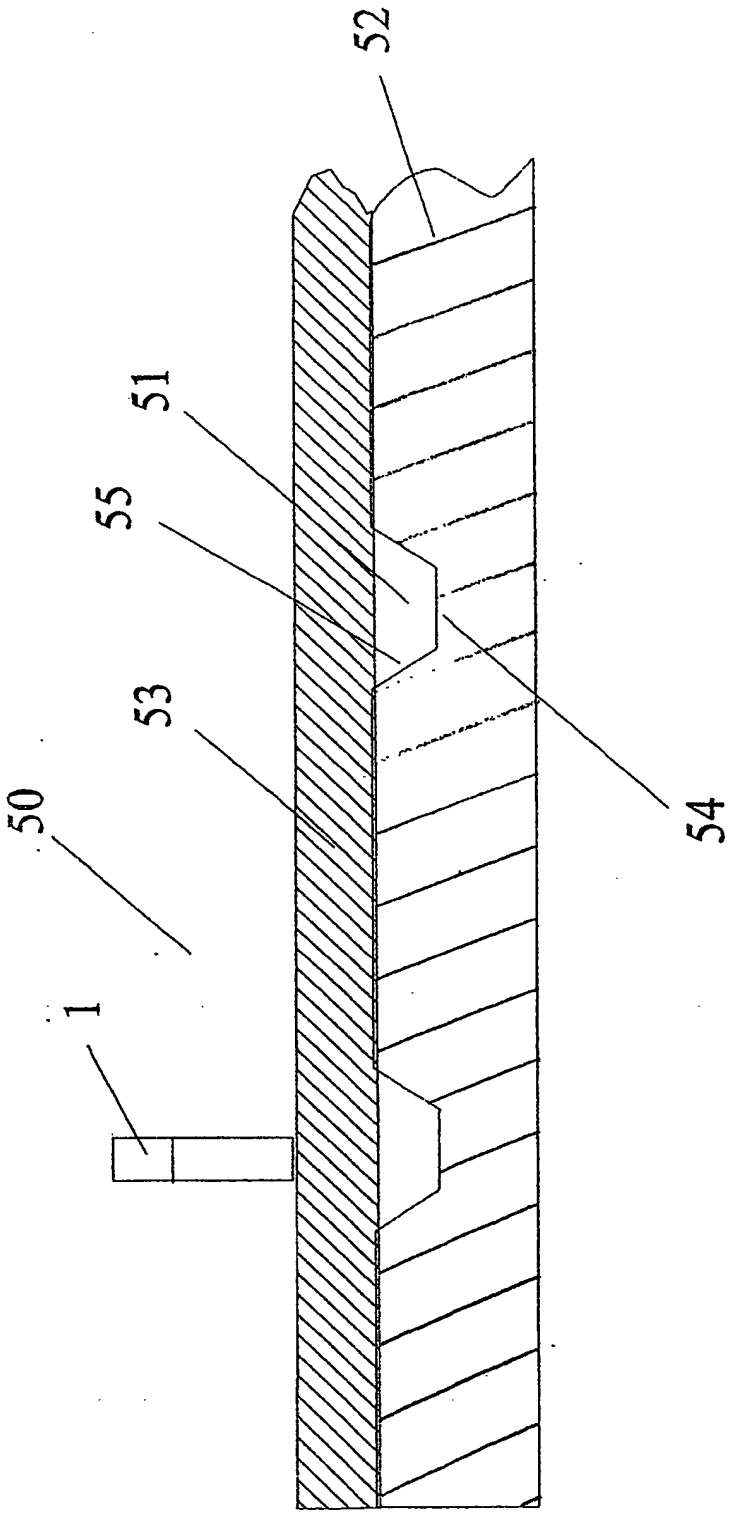


Fig. 2

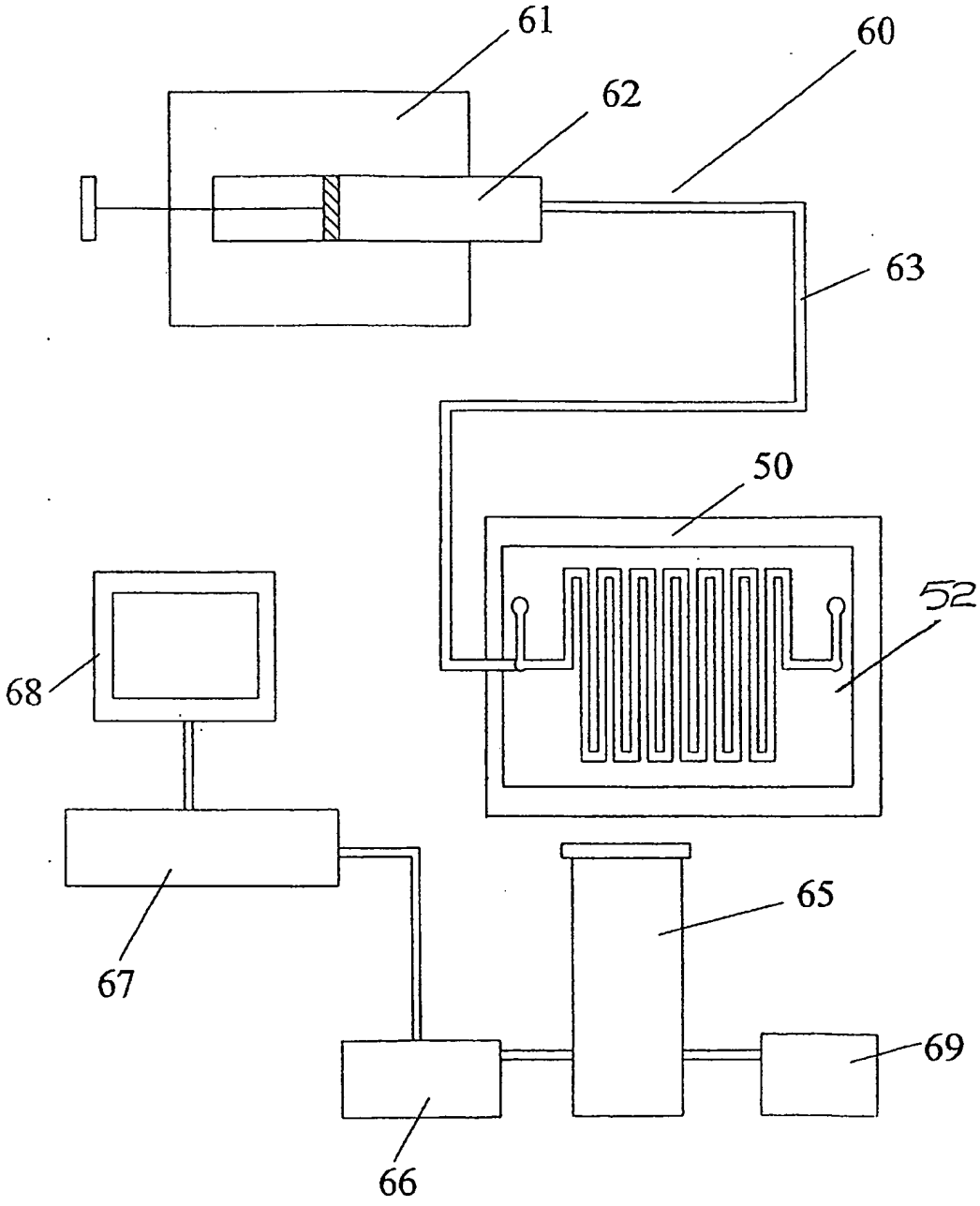
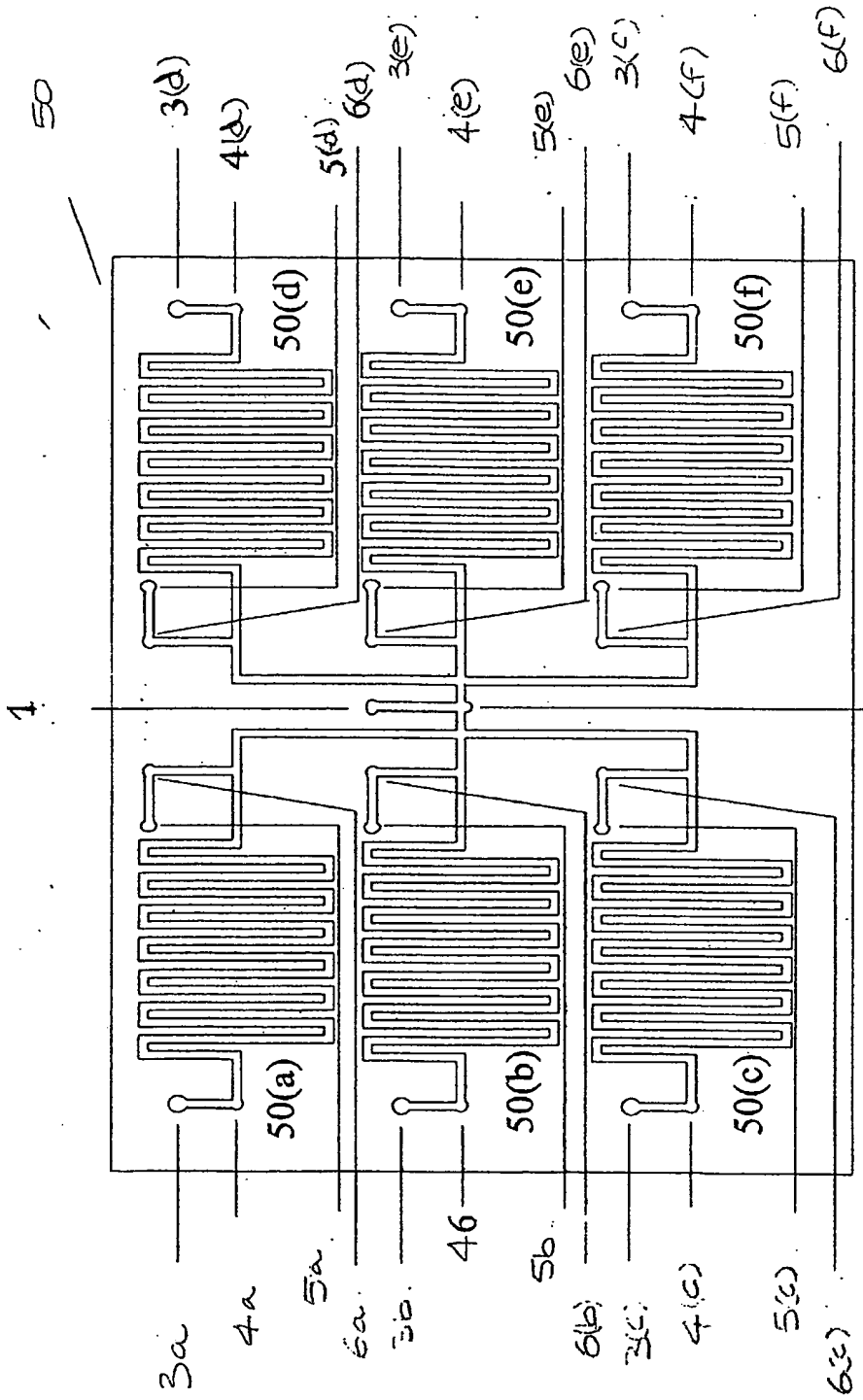


FIG 3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6(f)
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50(e)
50(f)
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3(c)
4(c)
5(c)
6(c)

FIG.4

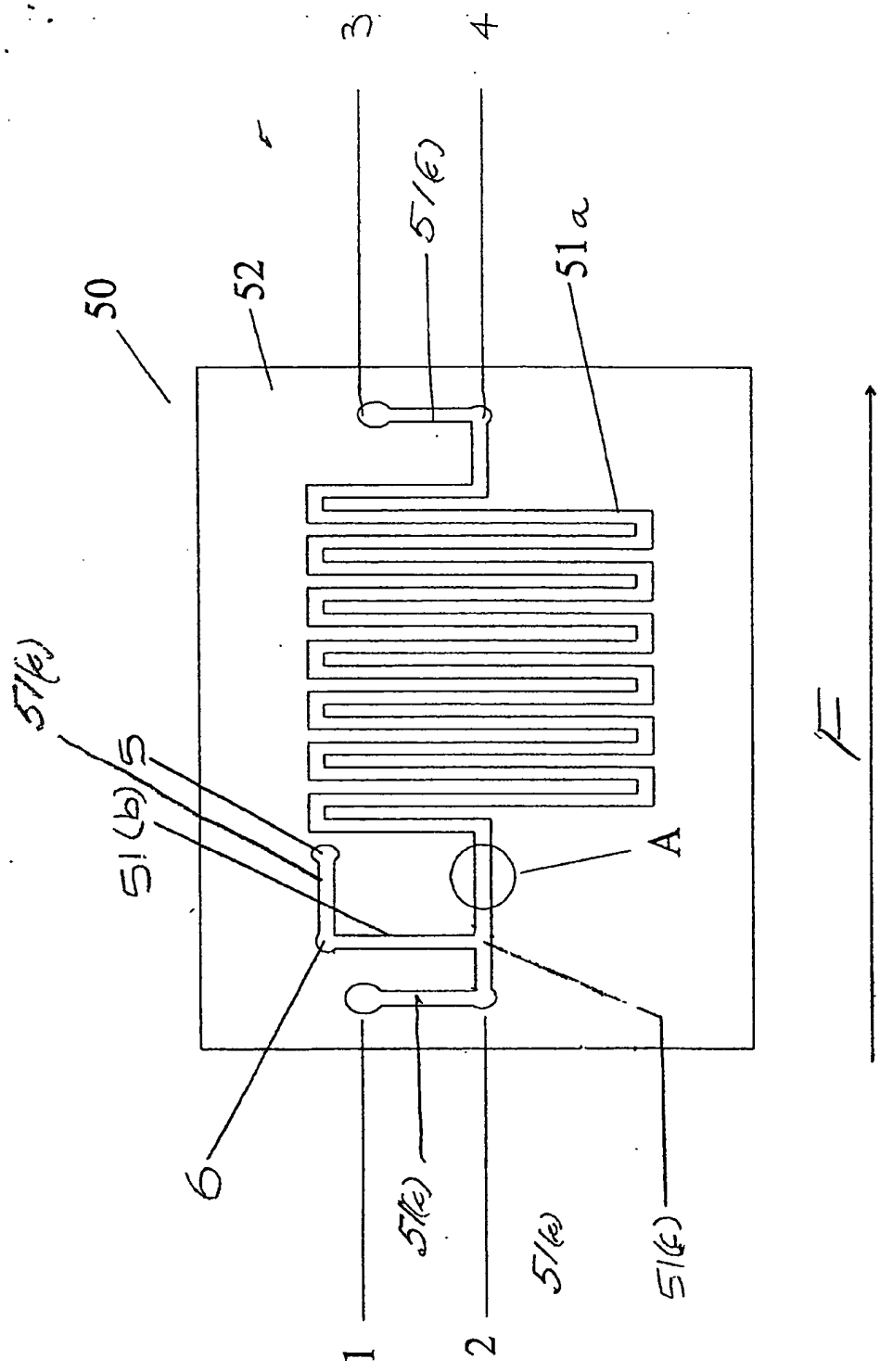


Fig. 5

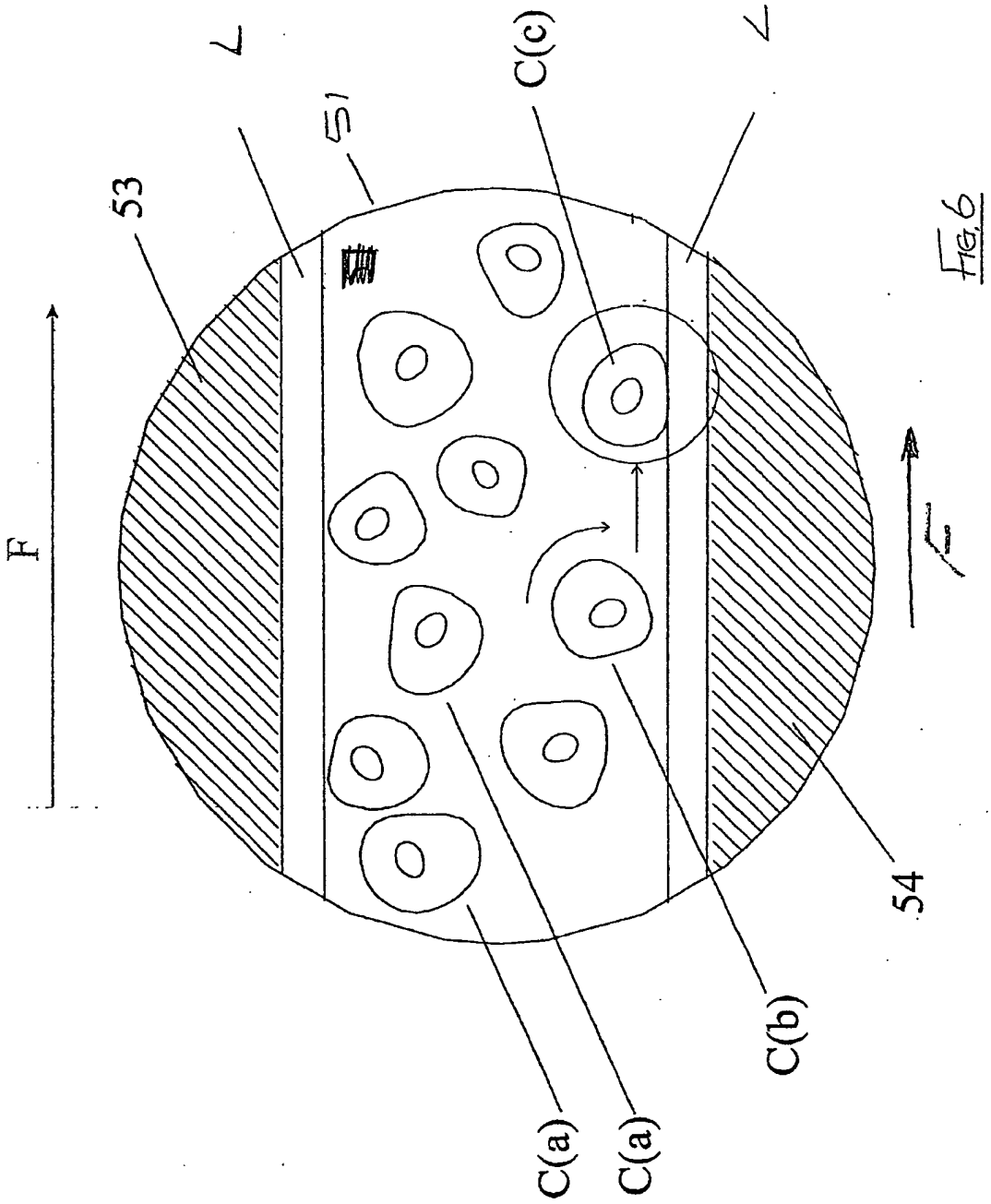


Fig. 6

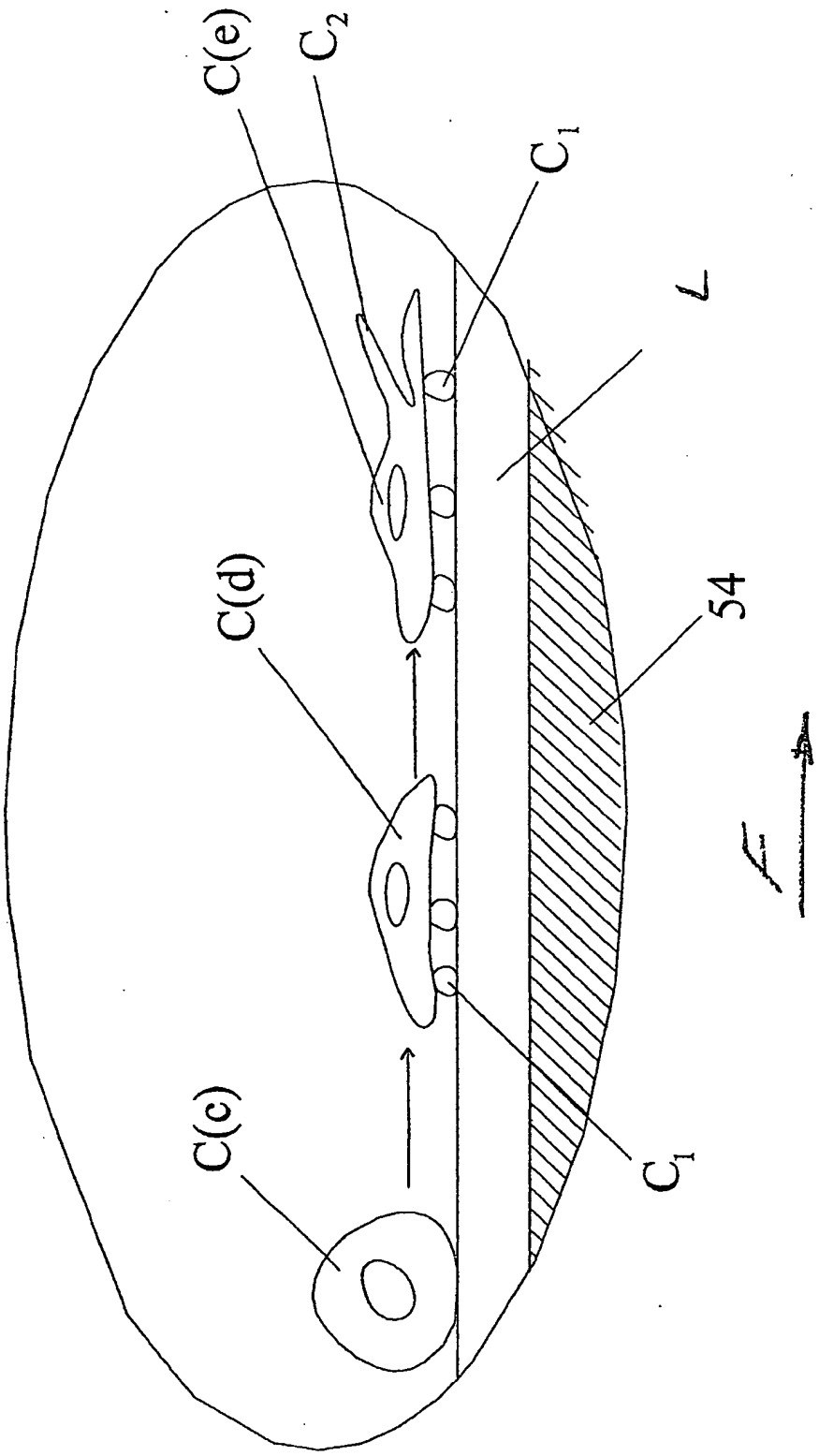


Fig 7

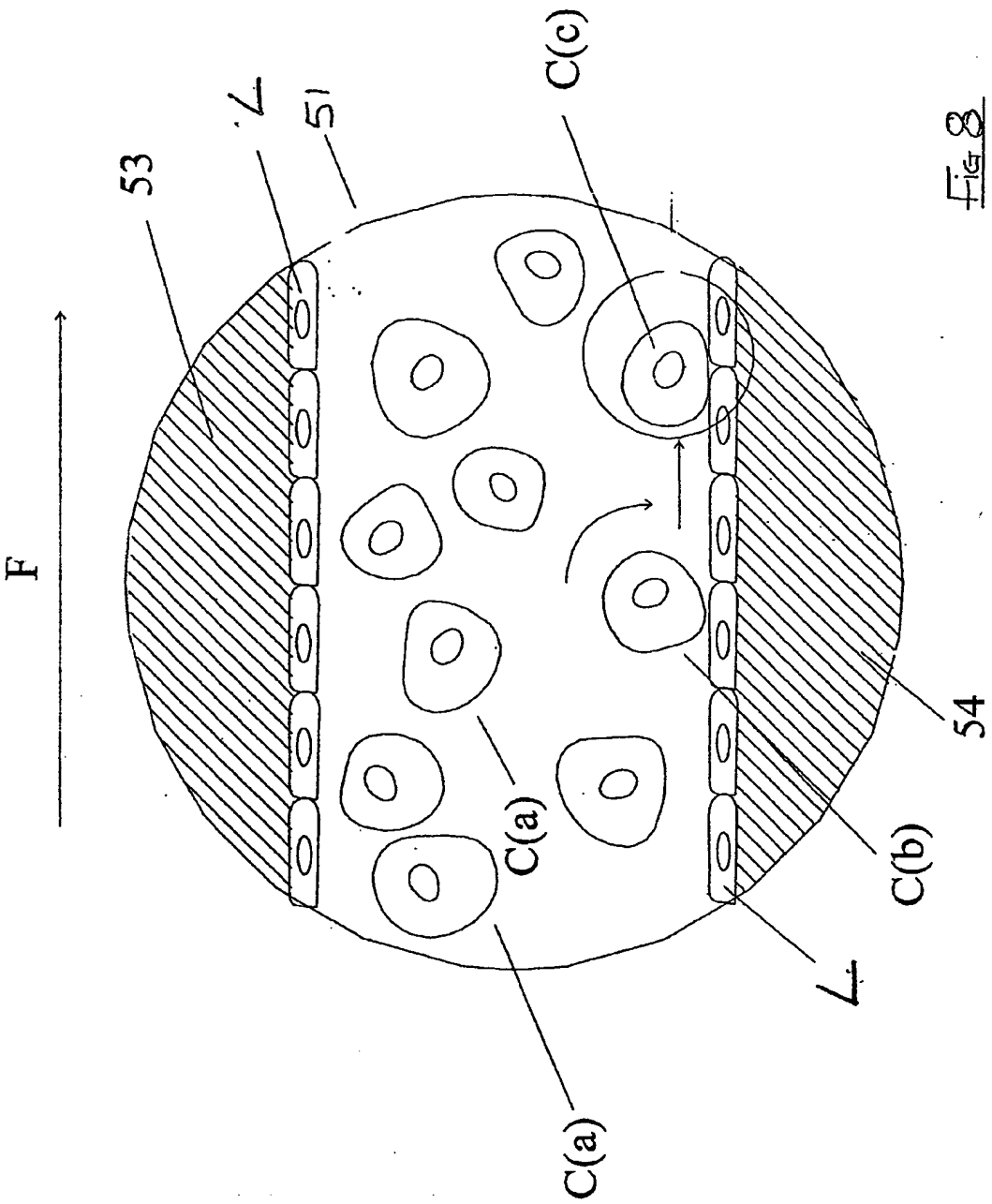


Fig 8

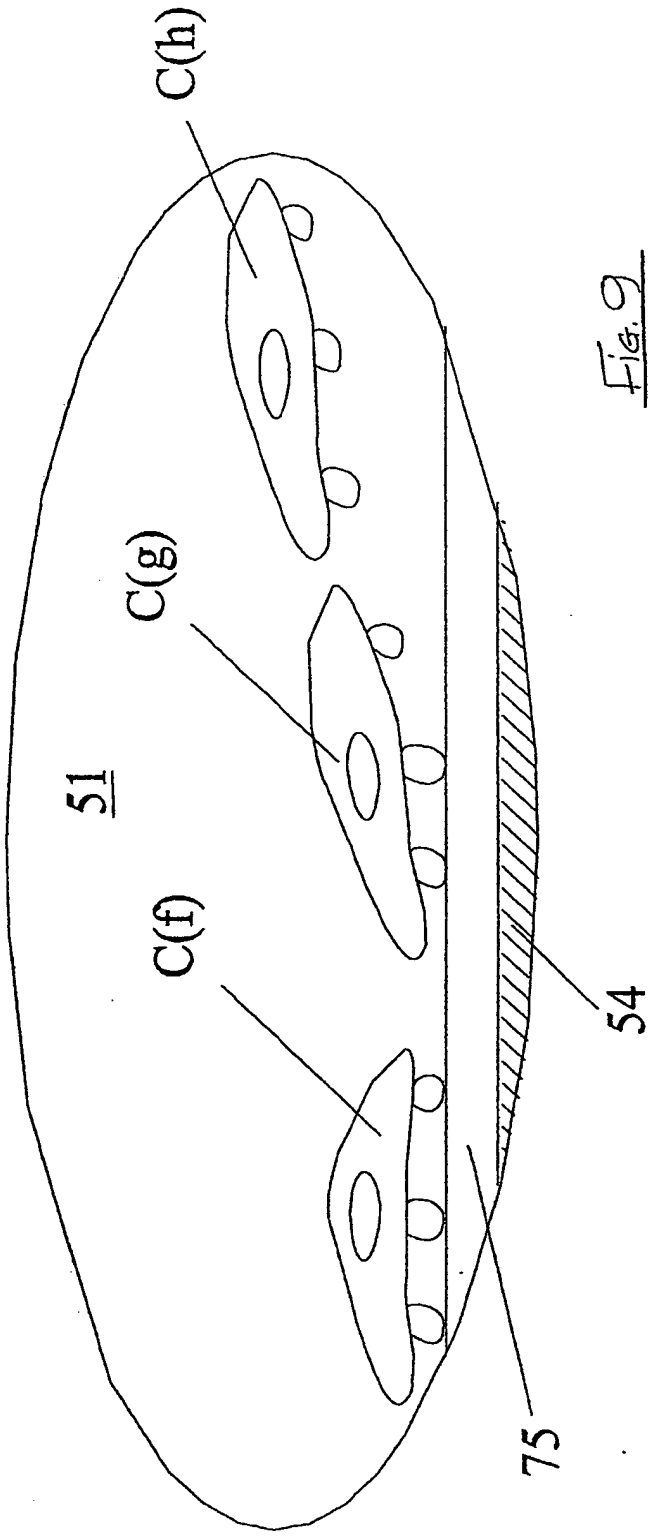


Fig. 9

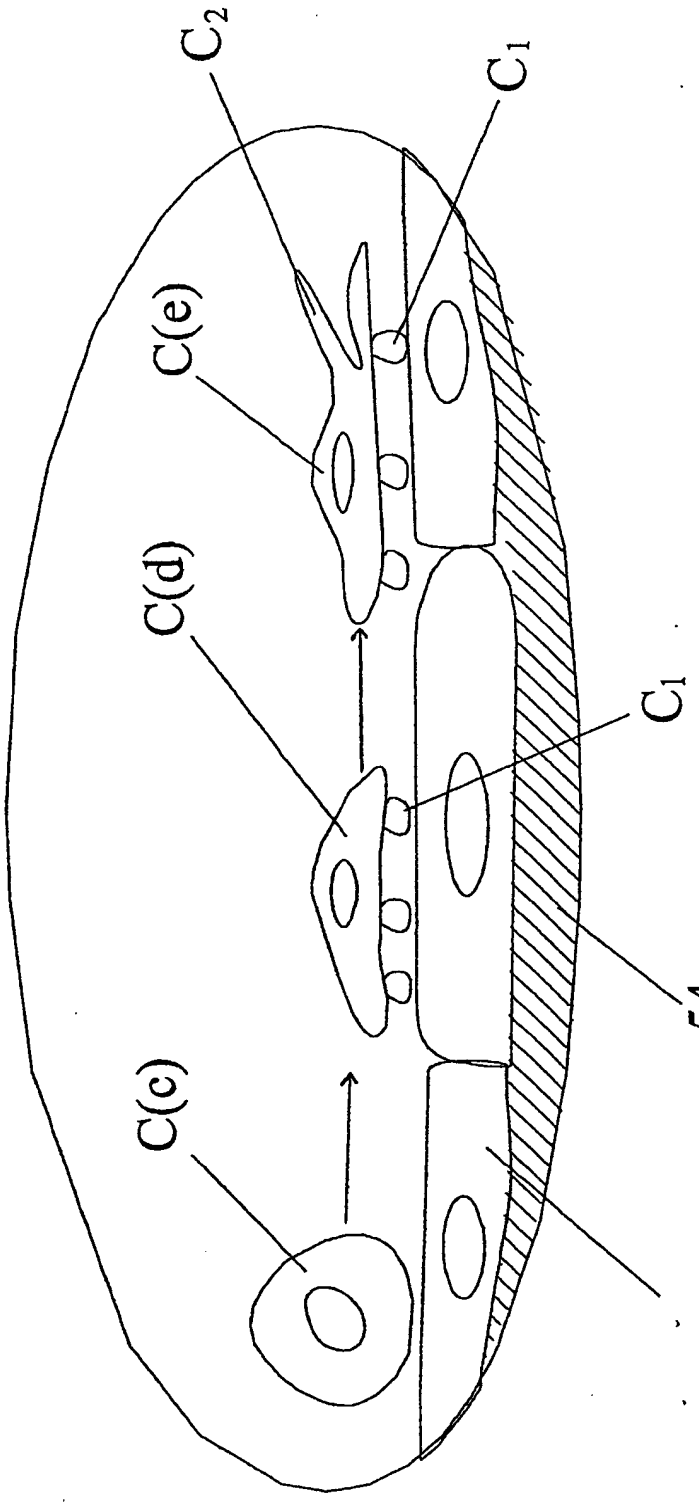
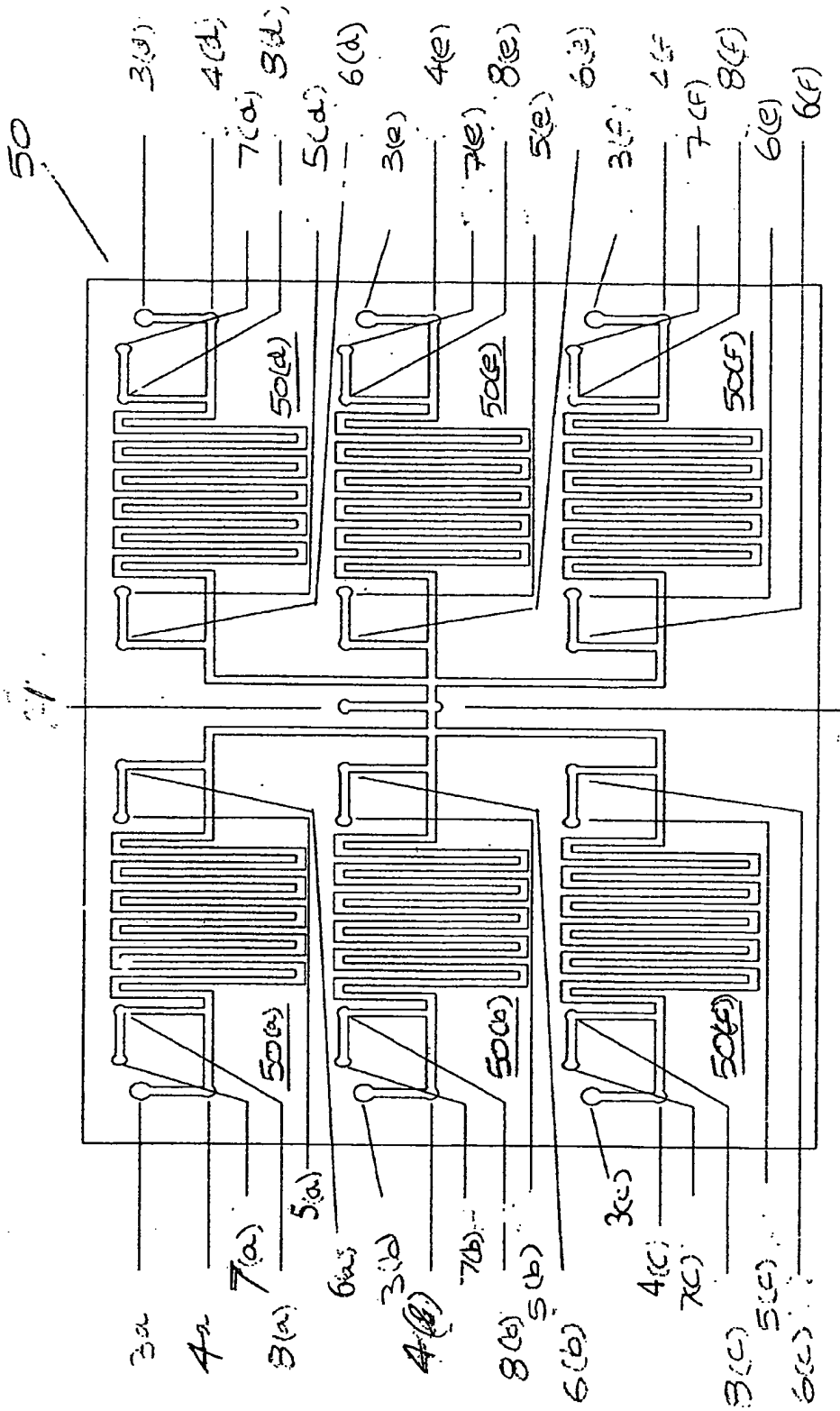


Fig. 10.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6(c)

Fig. 11

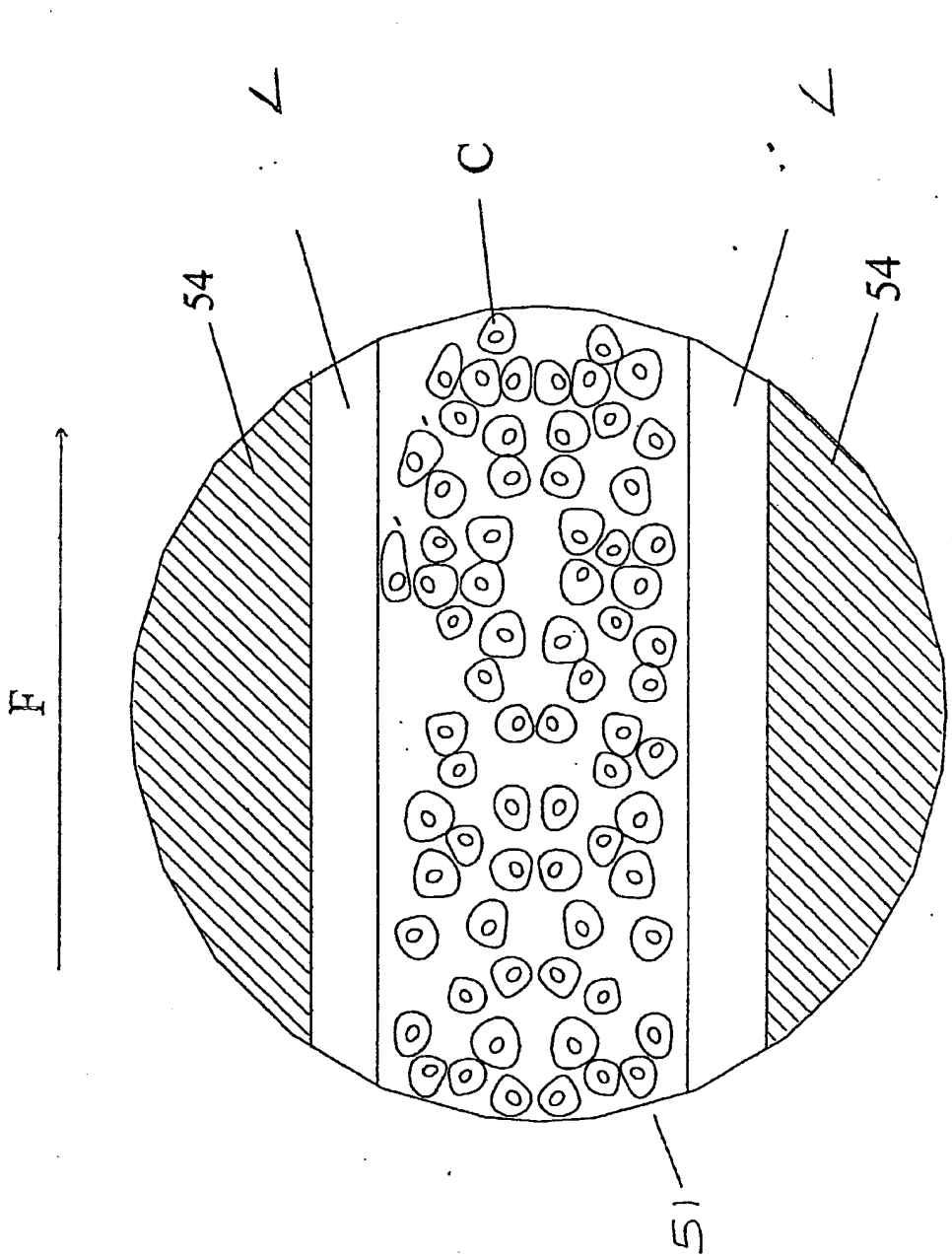

Fig 12

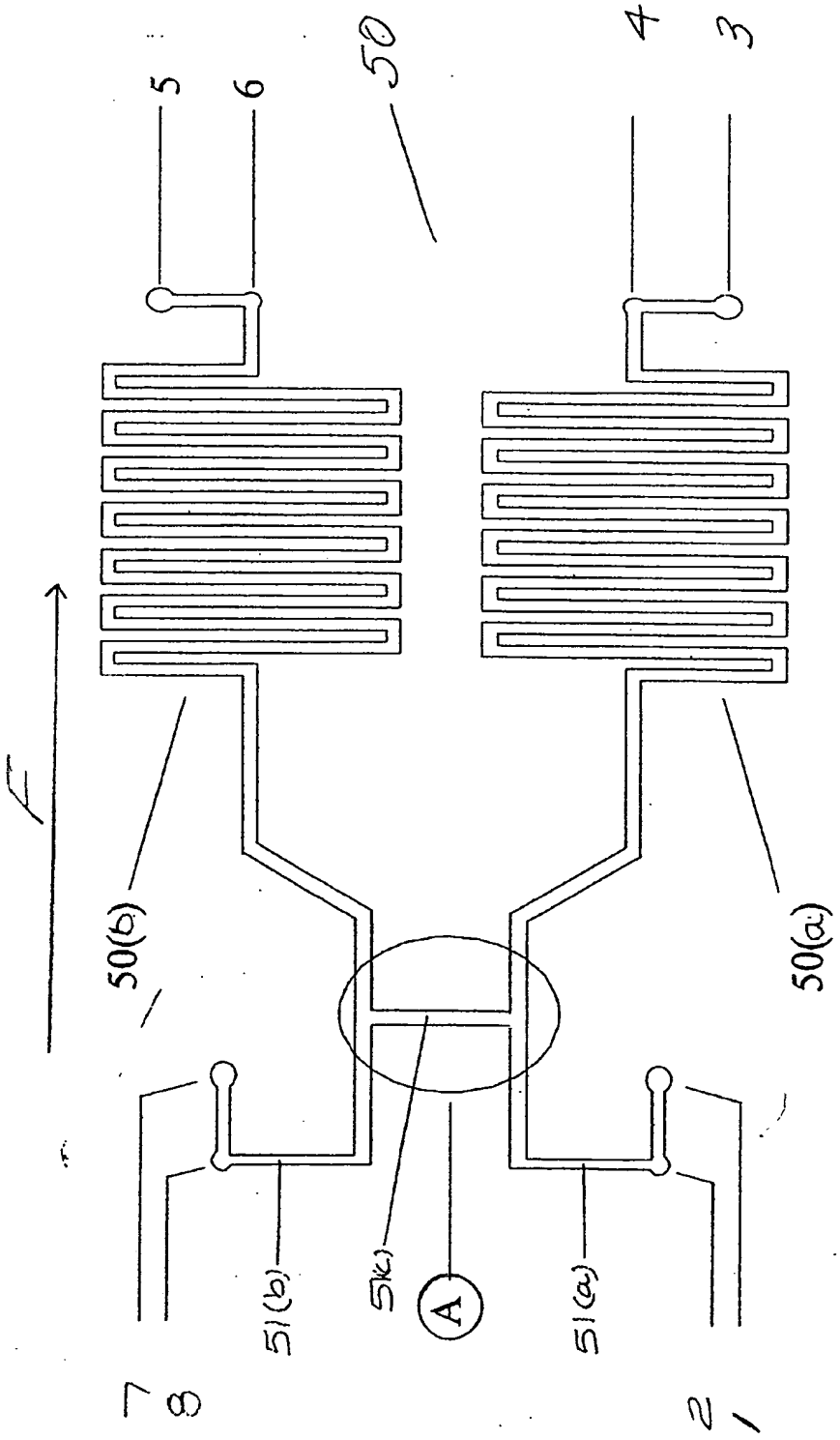

FIG 13

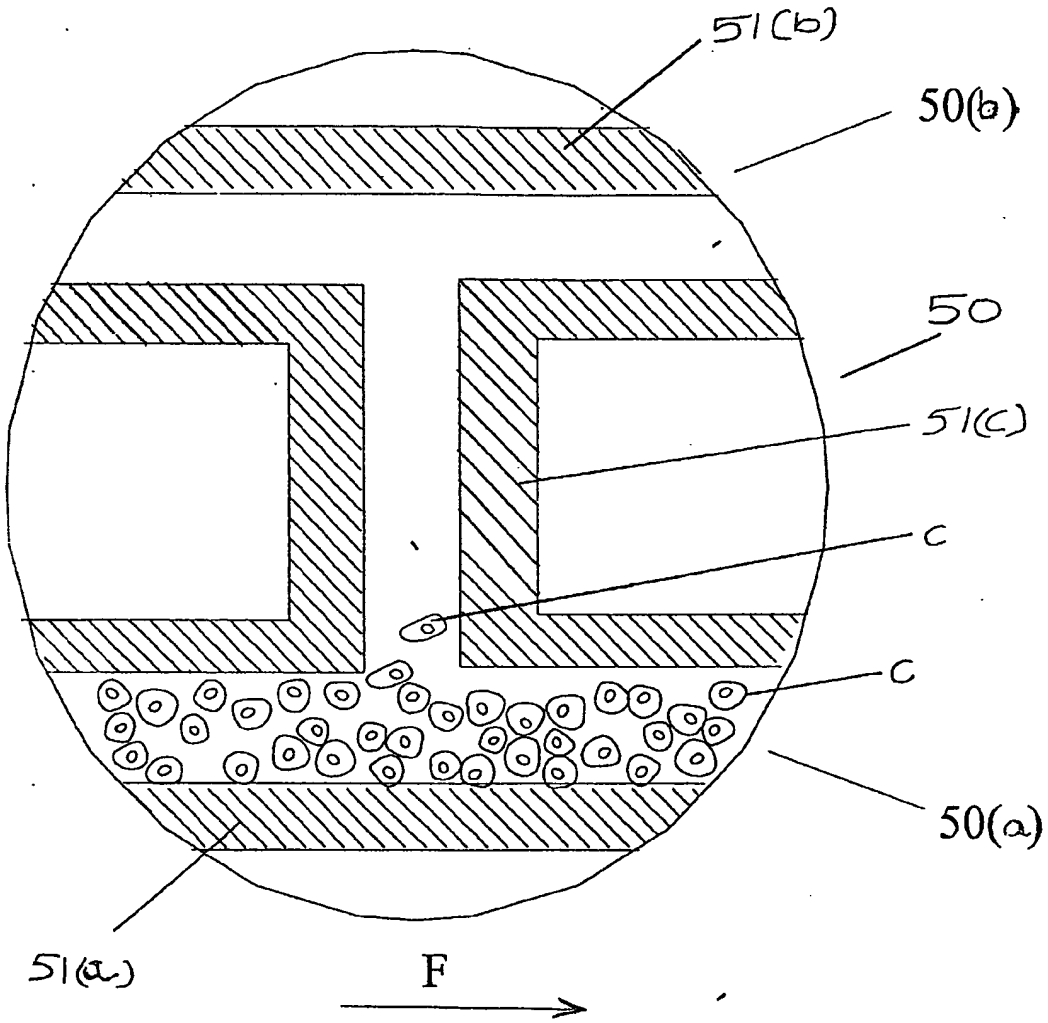

Fig 14

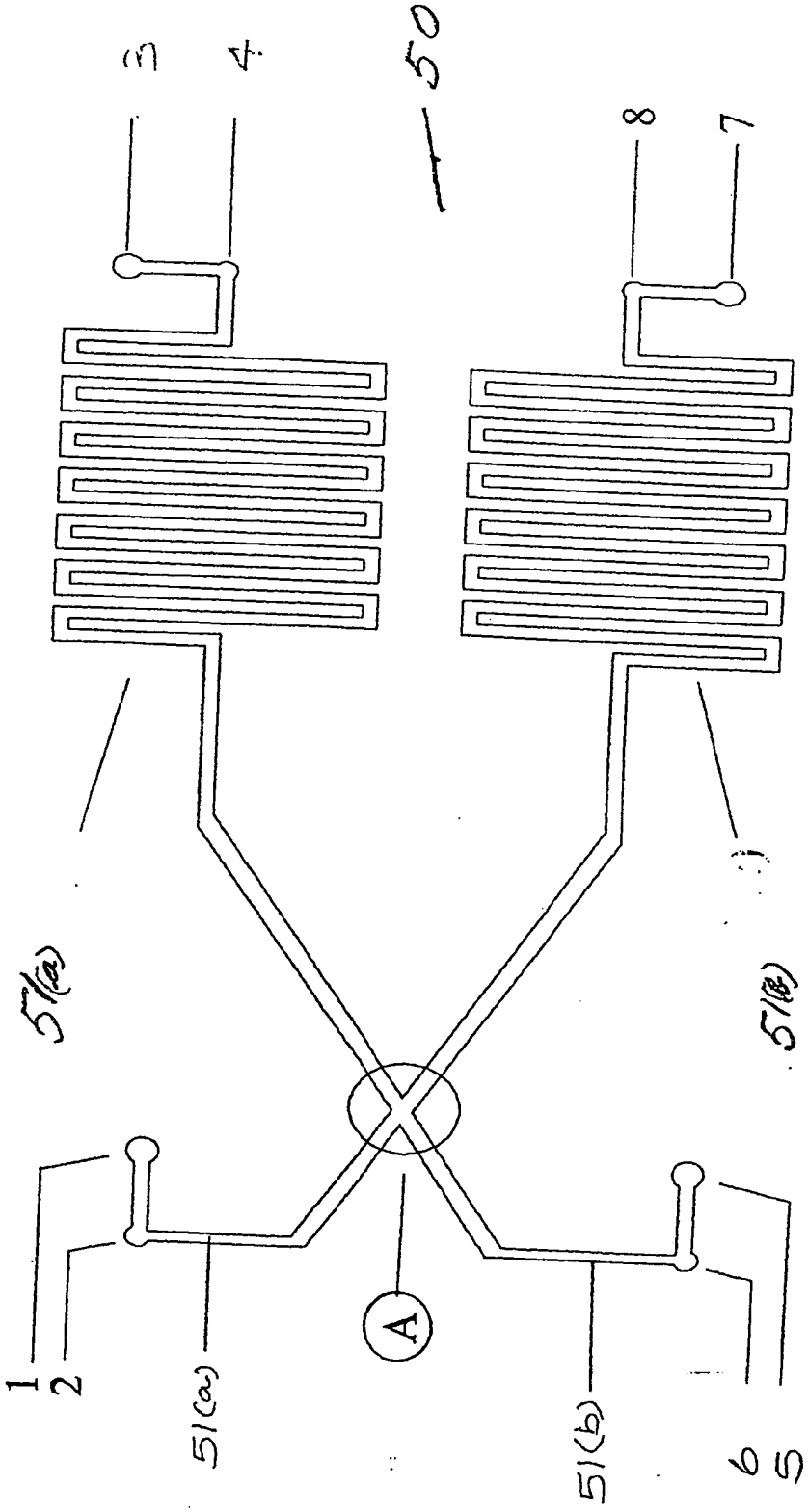

Fig 15

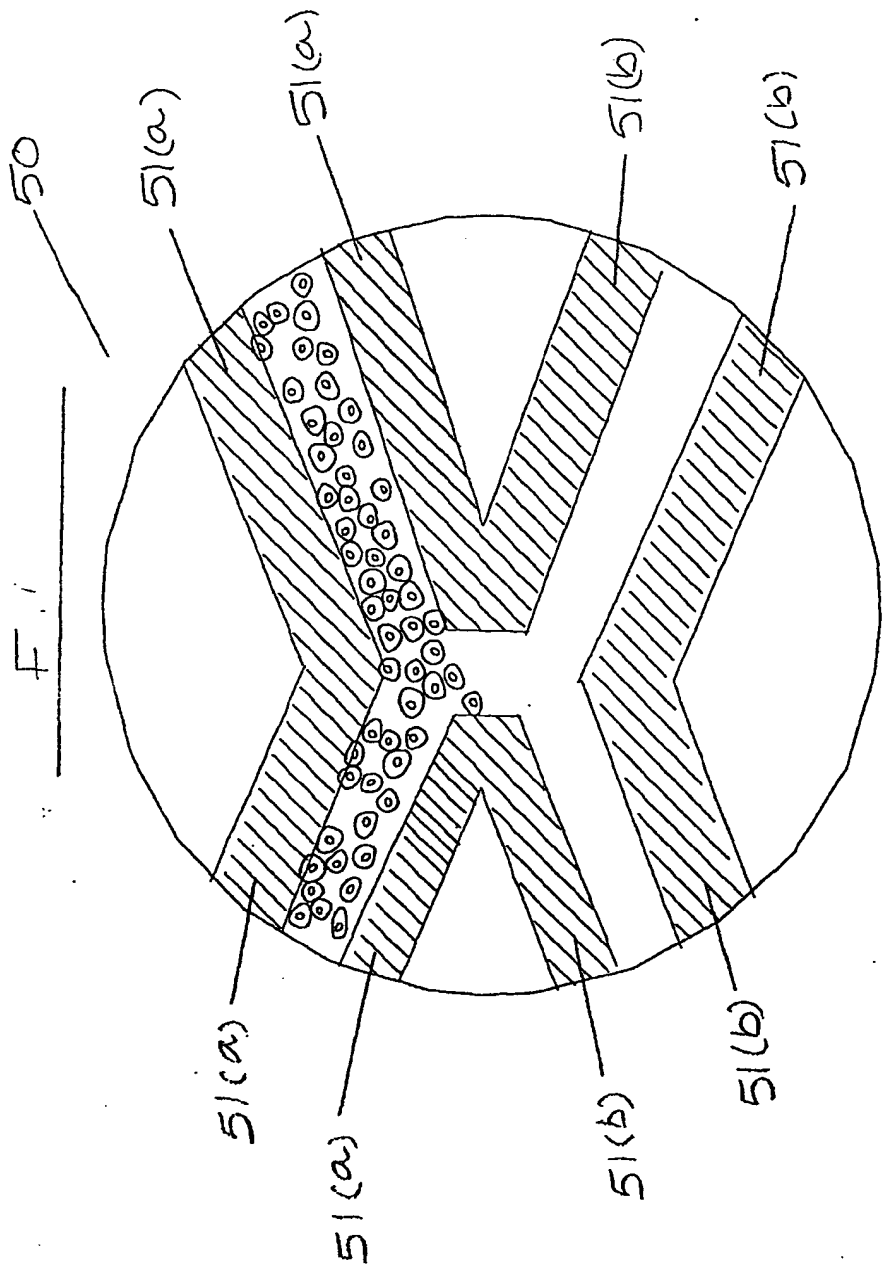

FIG. 16

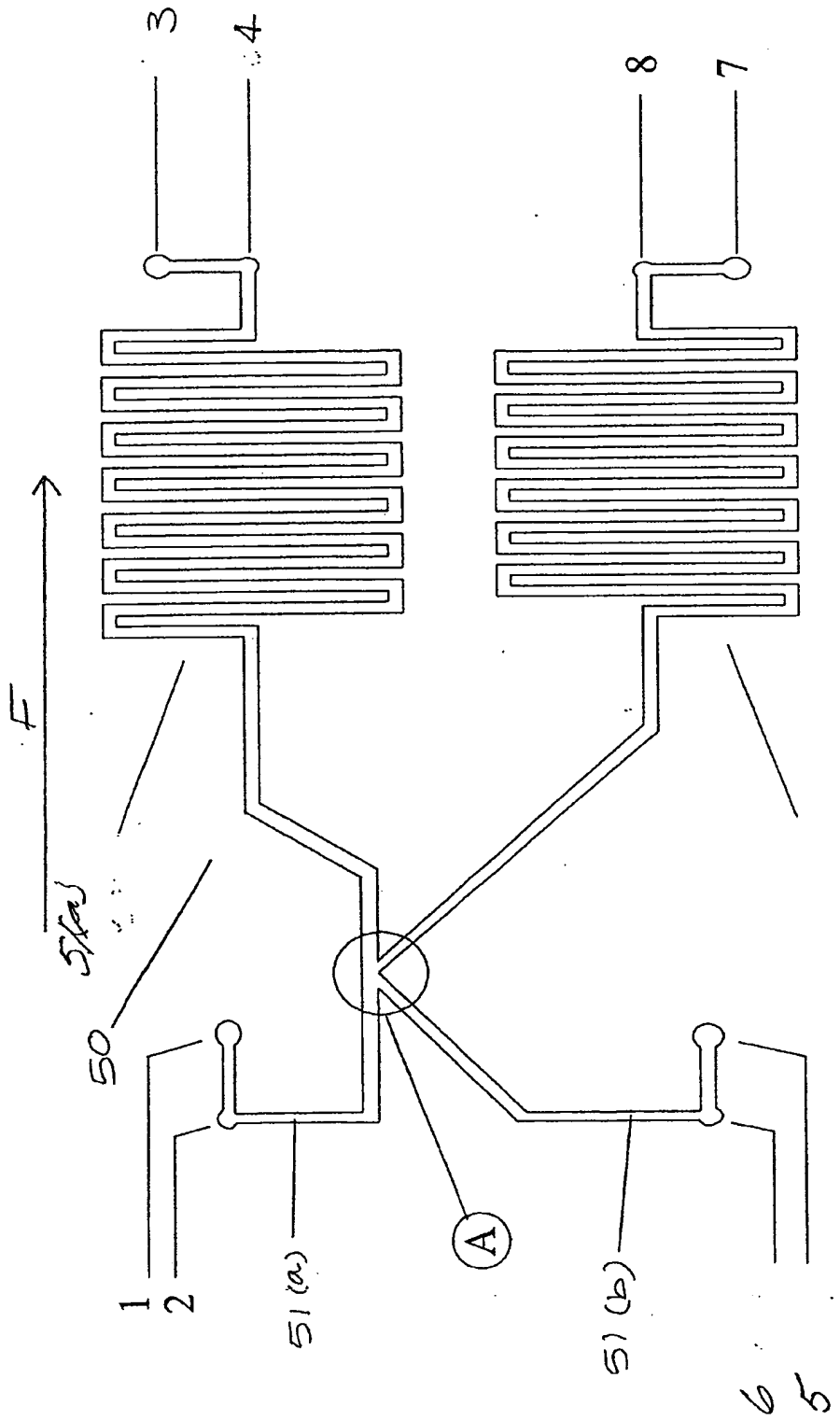

Fig.17

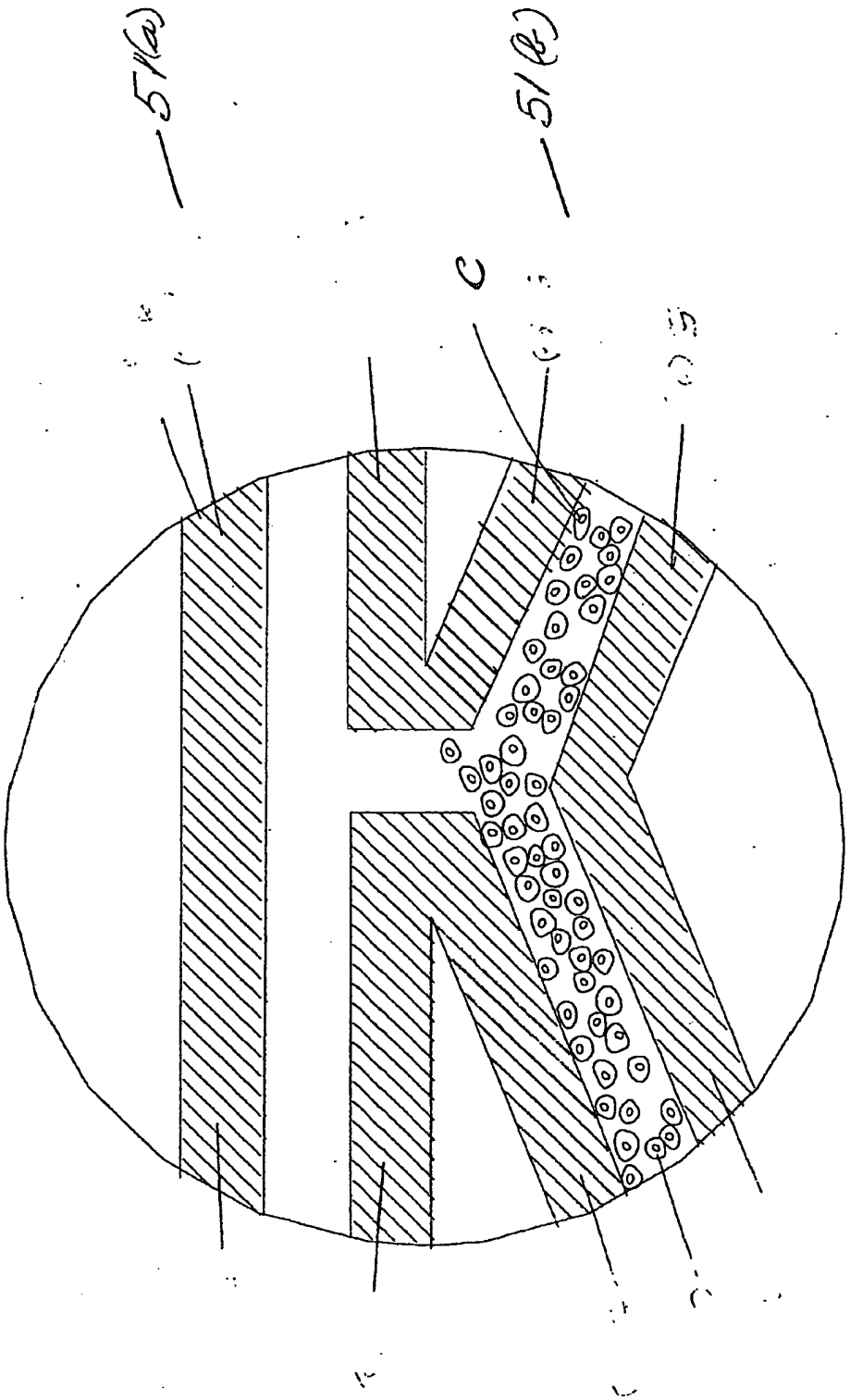

Fig 18

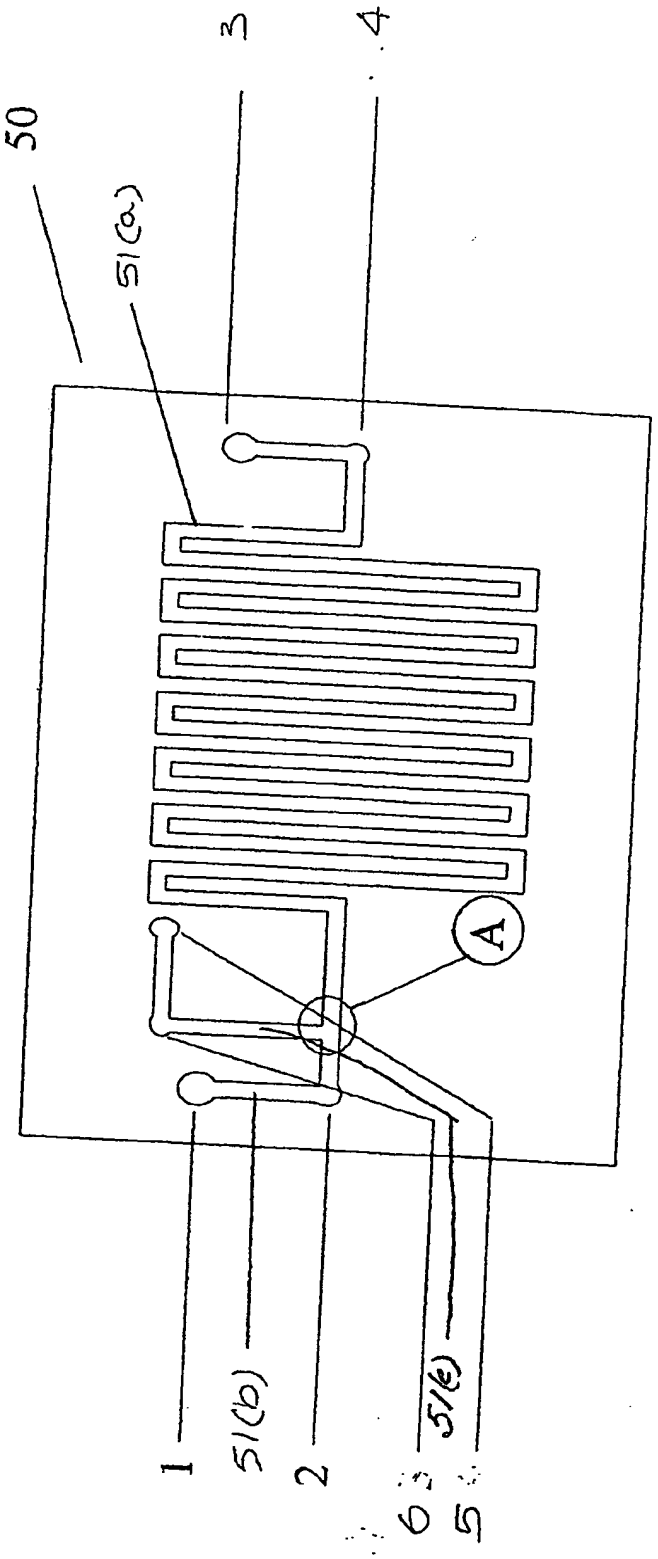

Fig. 19

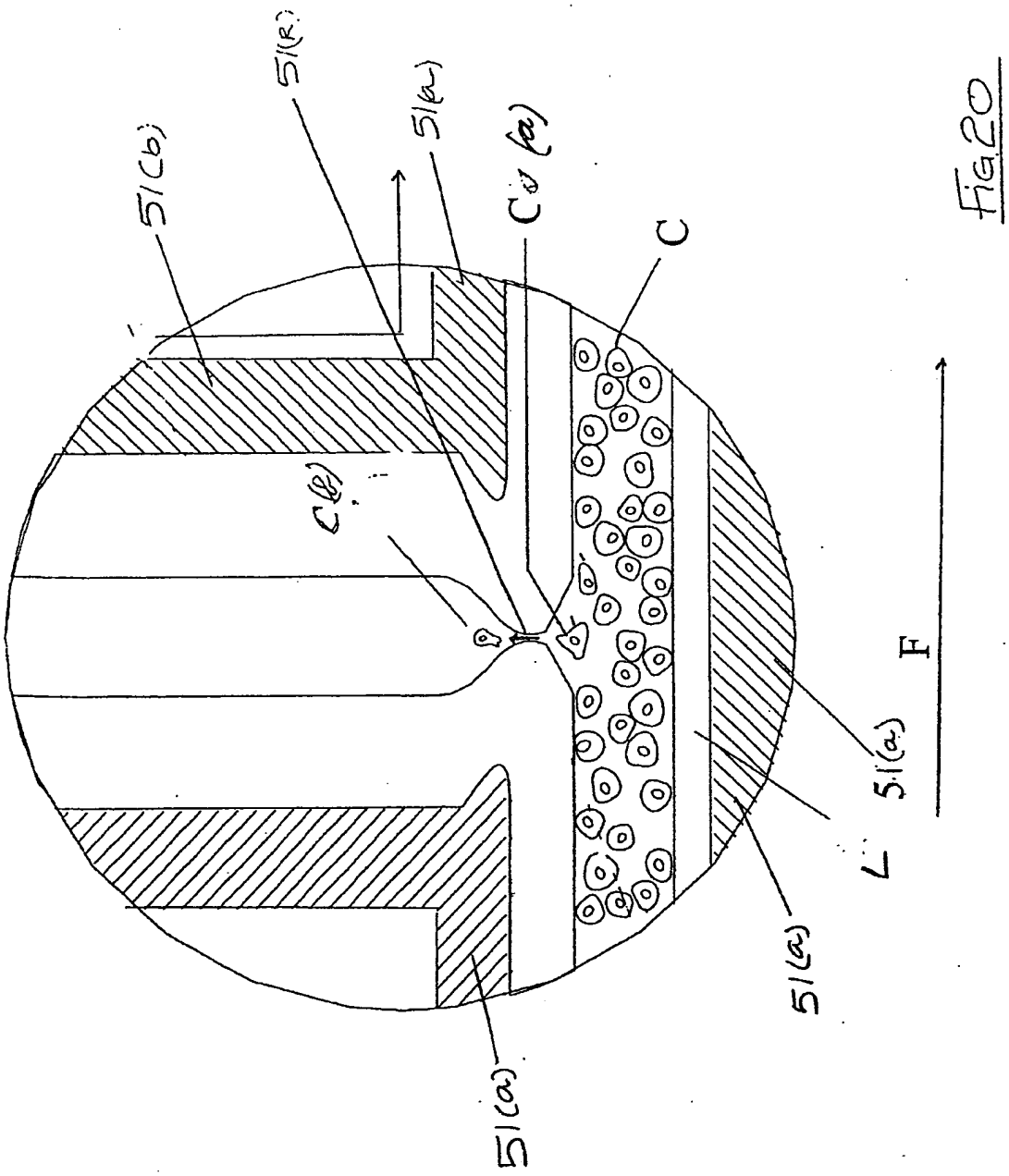

Fig.20

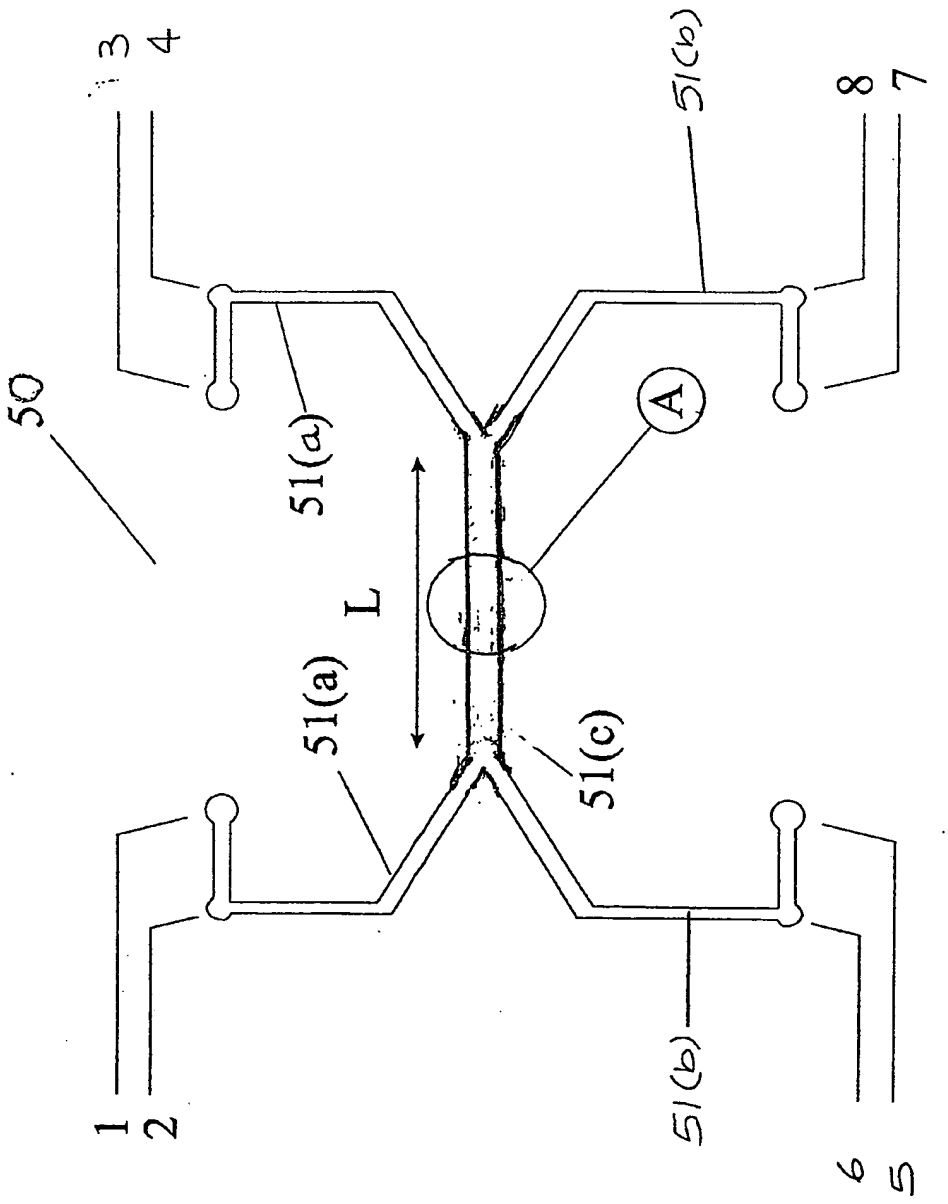

Fig. 21

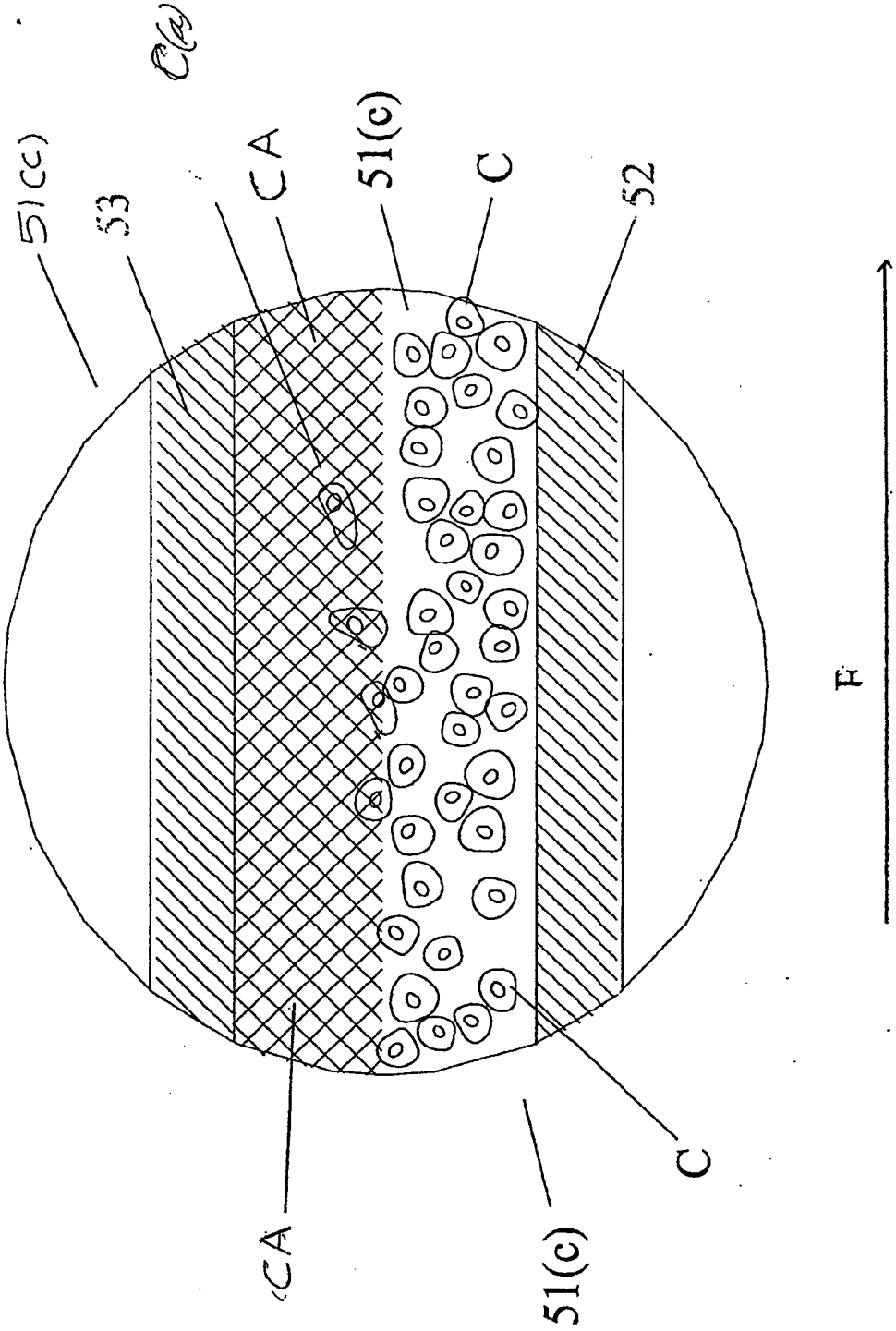

Fig.22

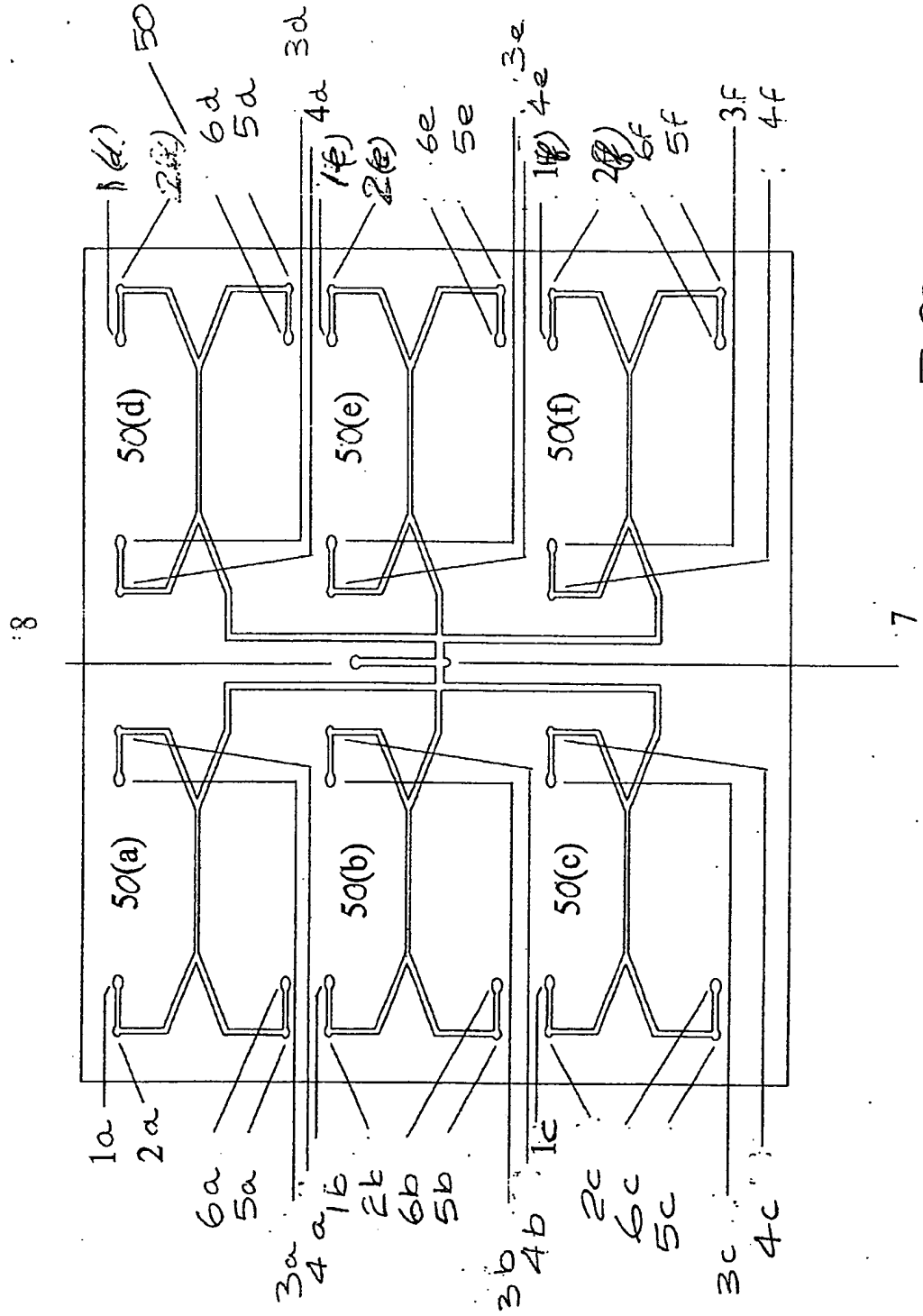
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Fig 23

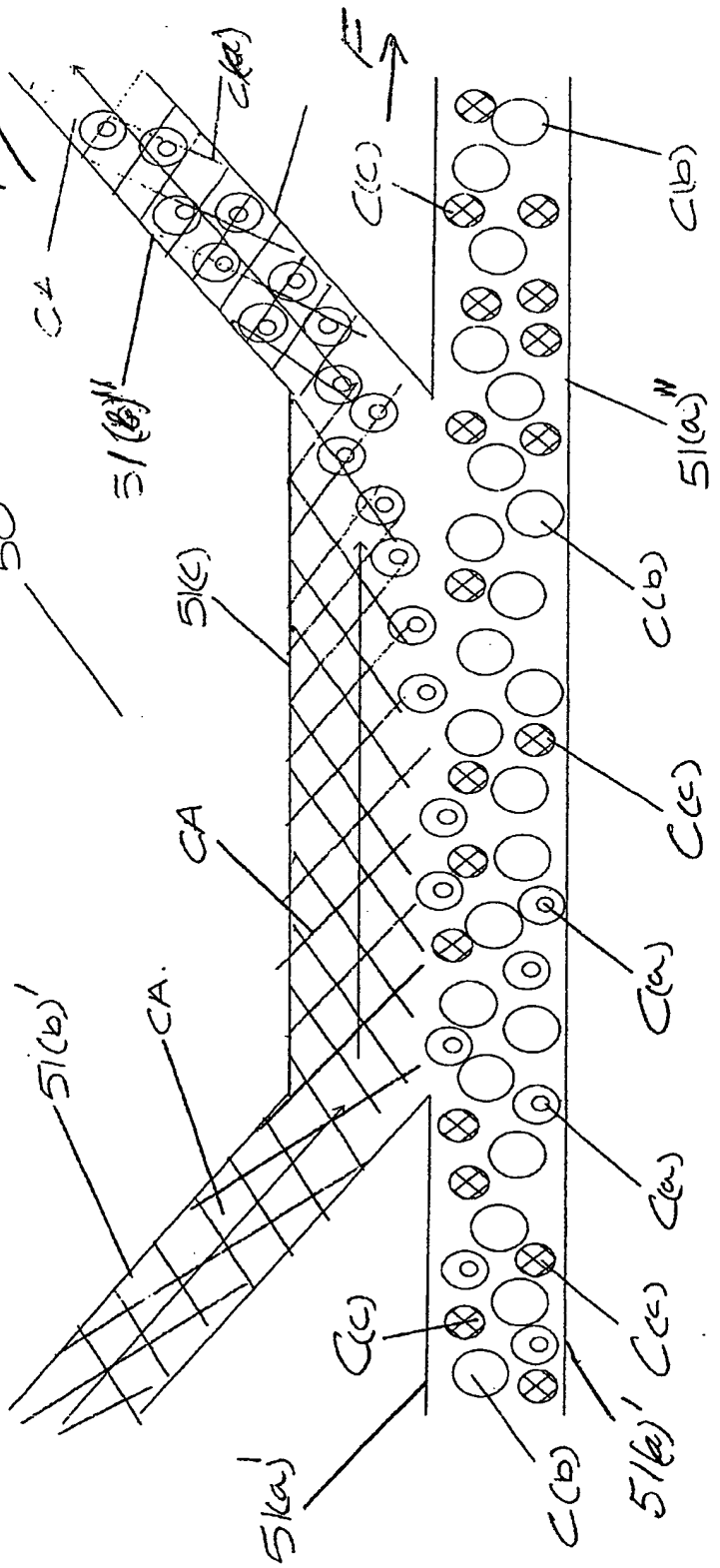

Fig 24

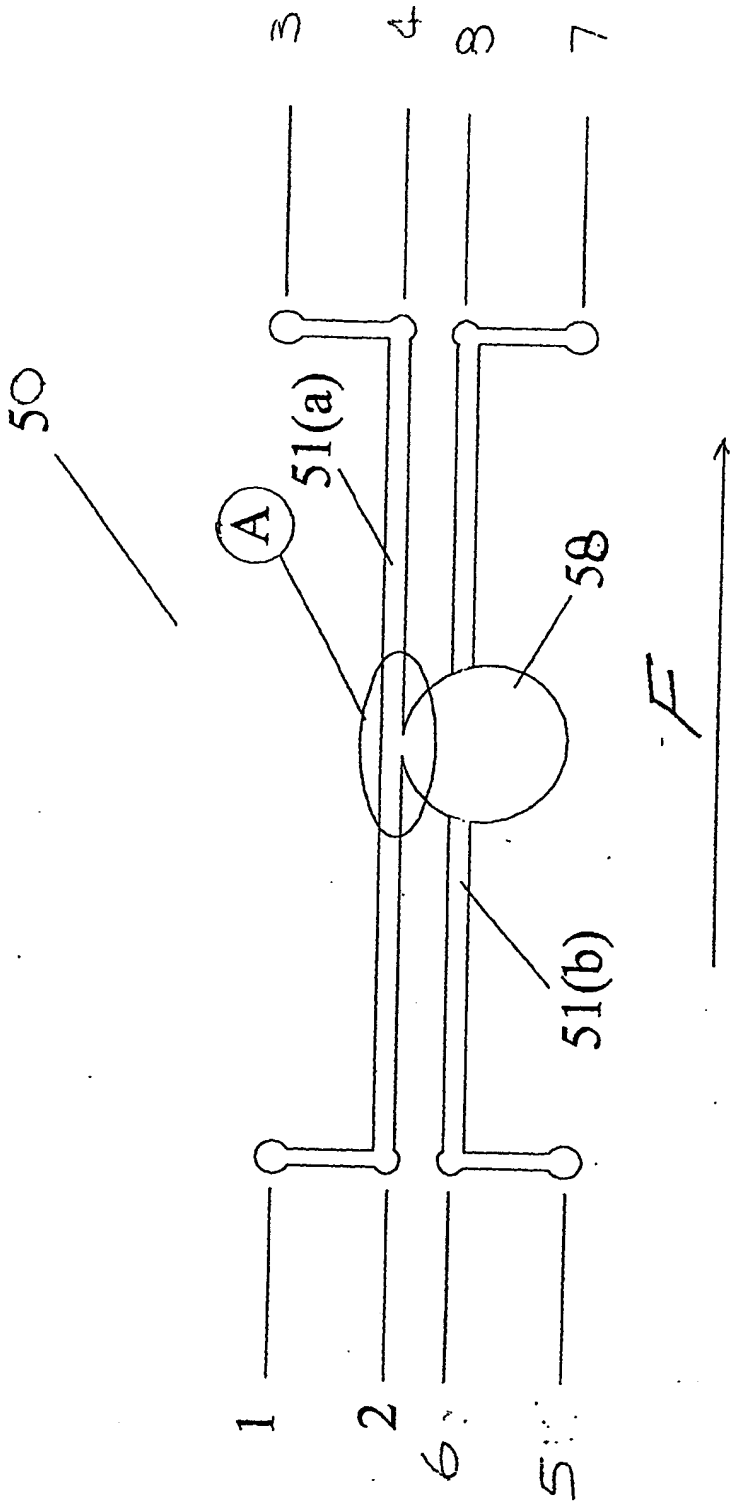

Fig.25

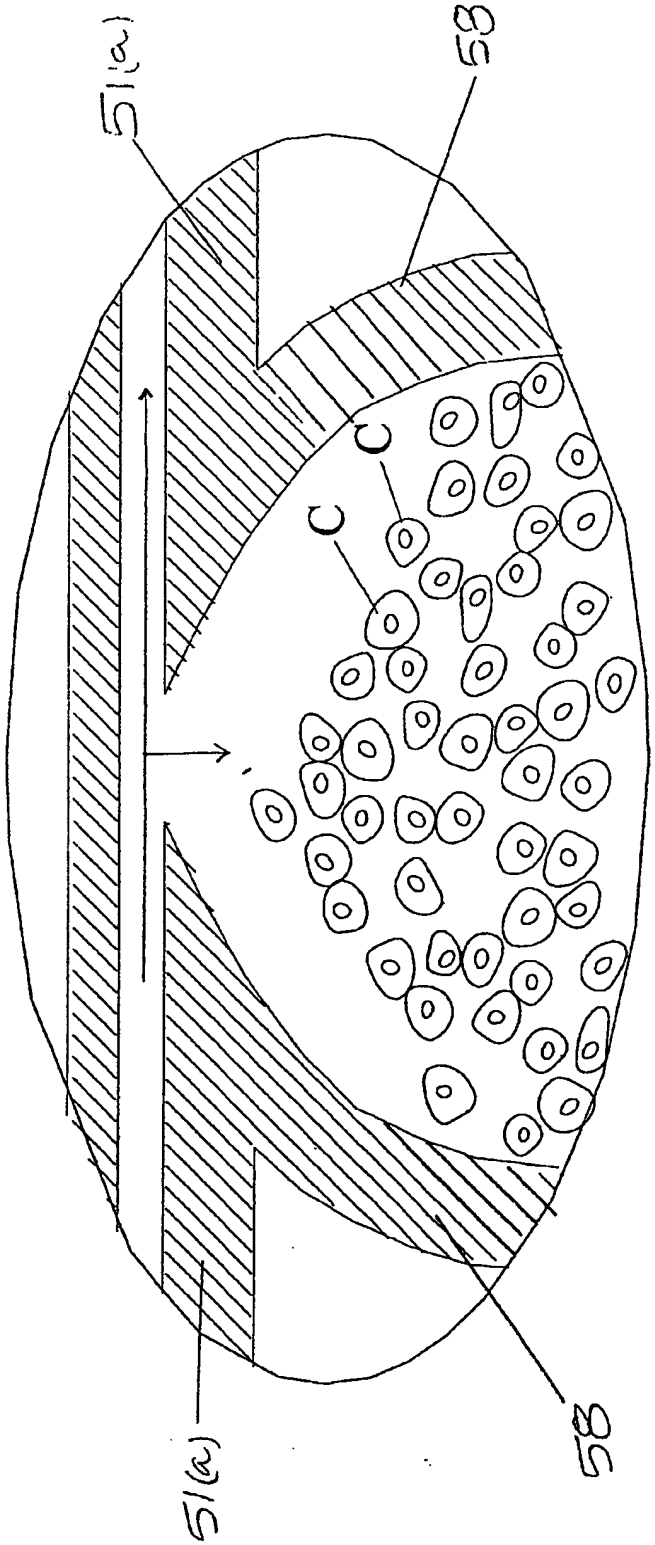

Fig.26

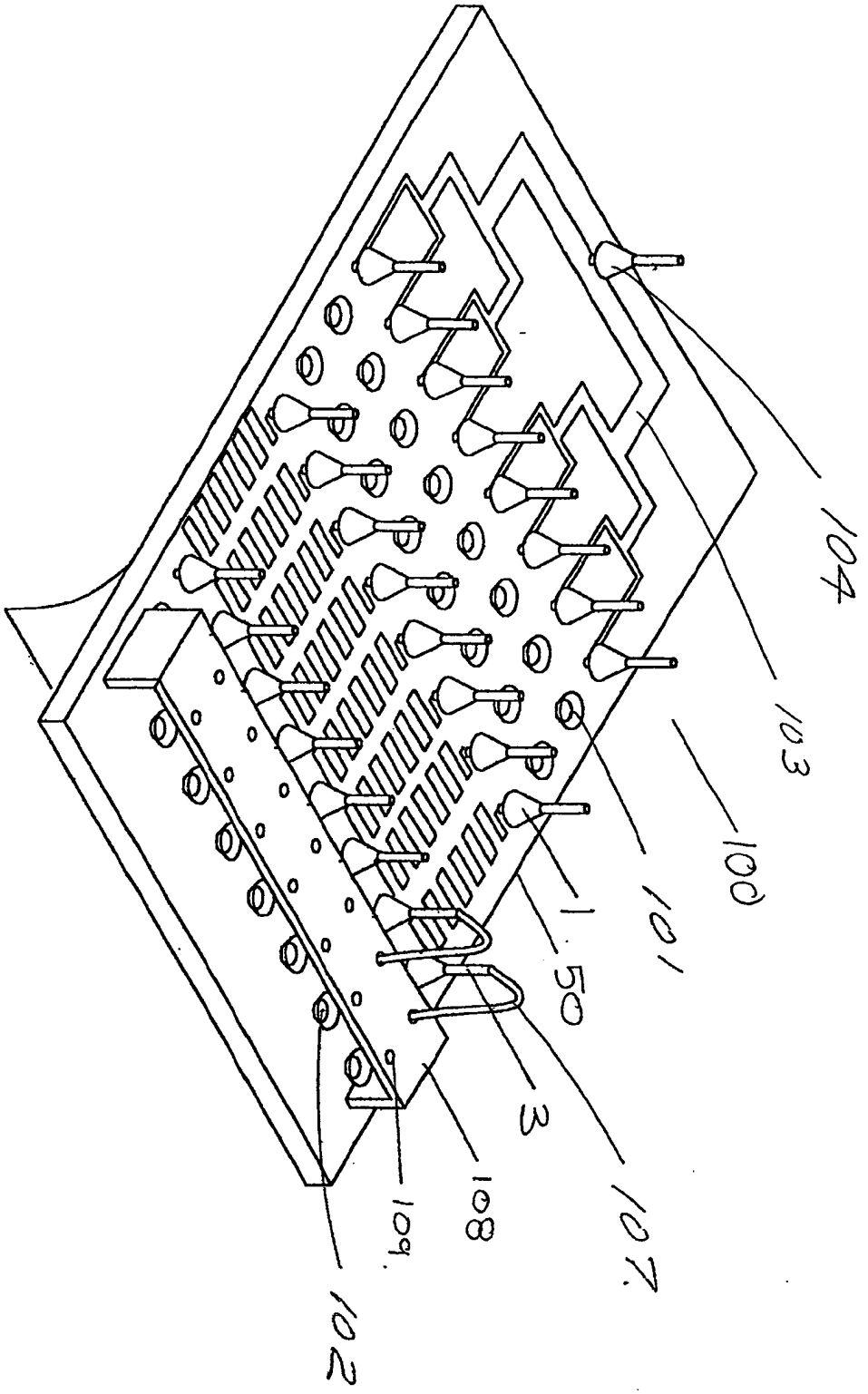

Fig 27

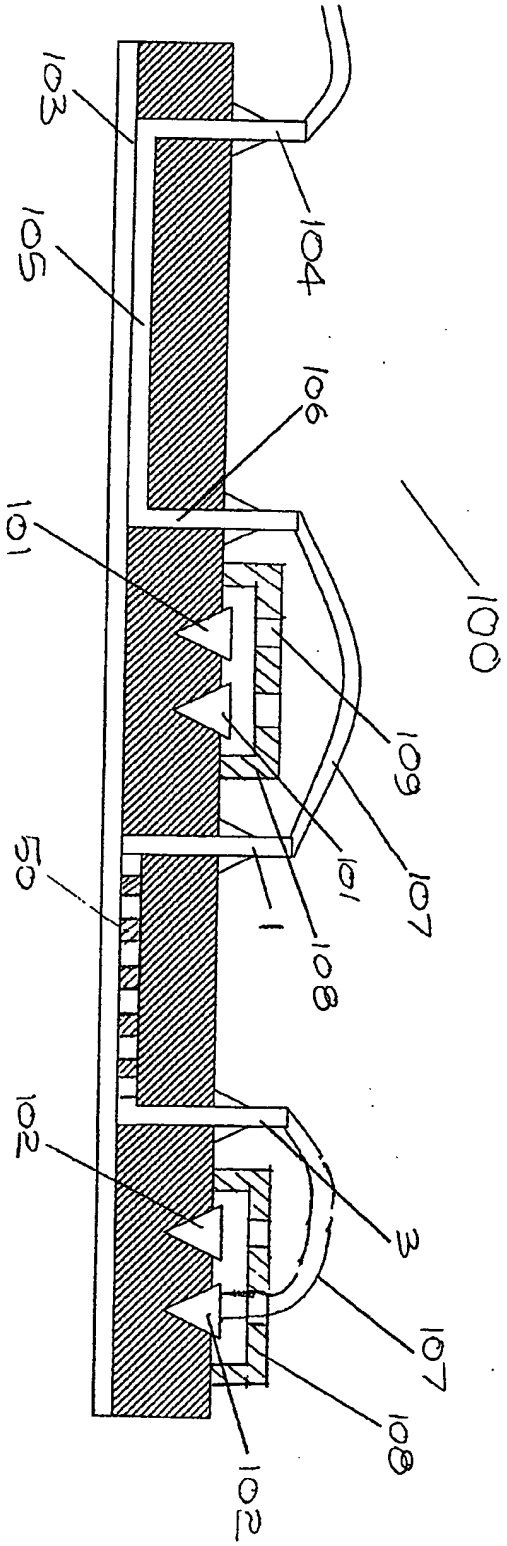

Fig.28

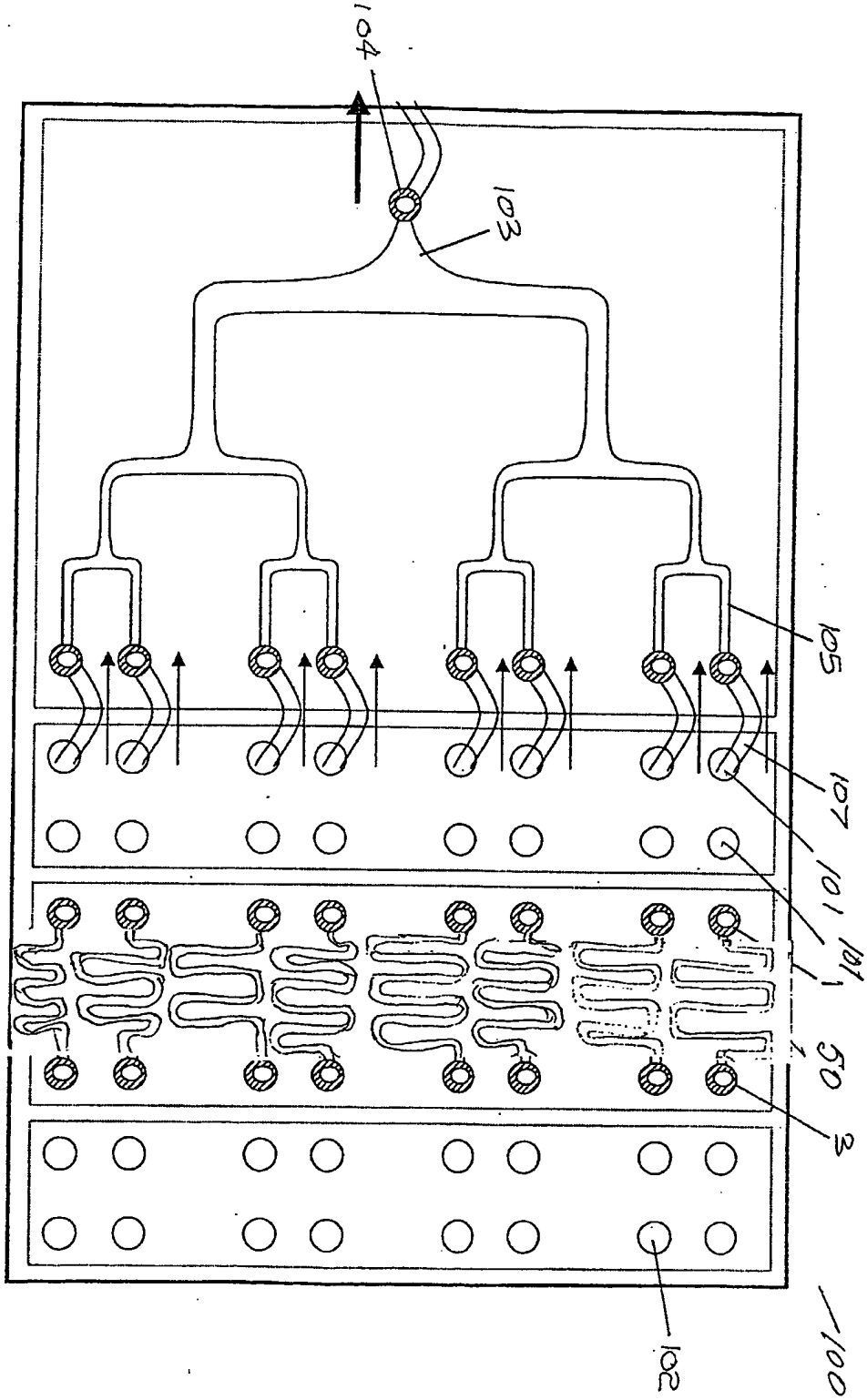

Fig. 29

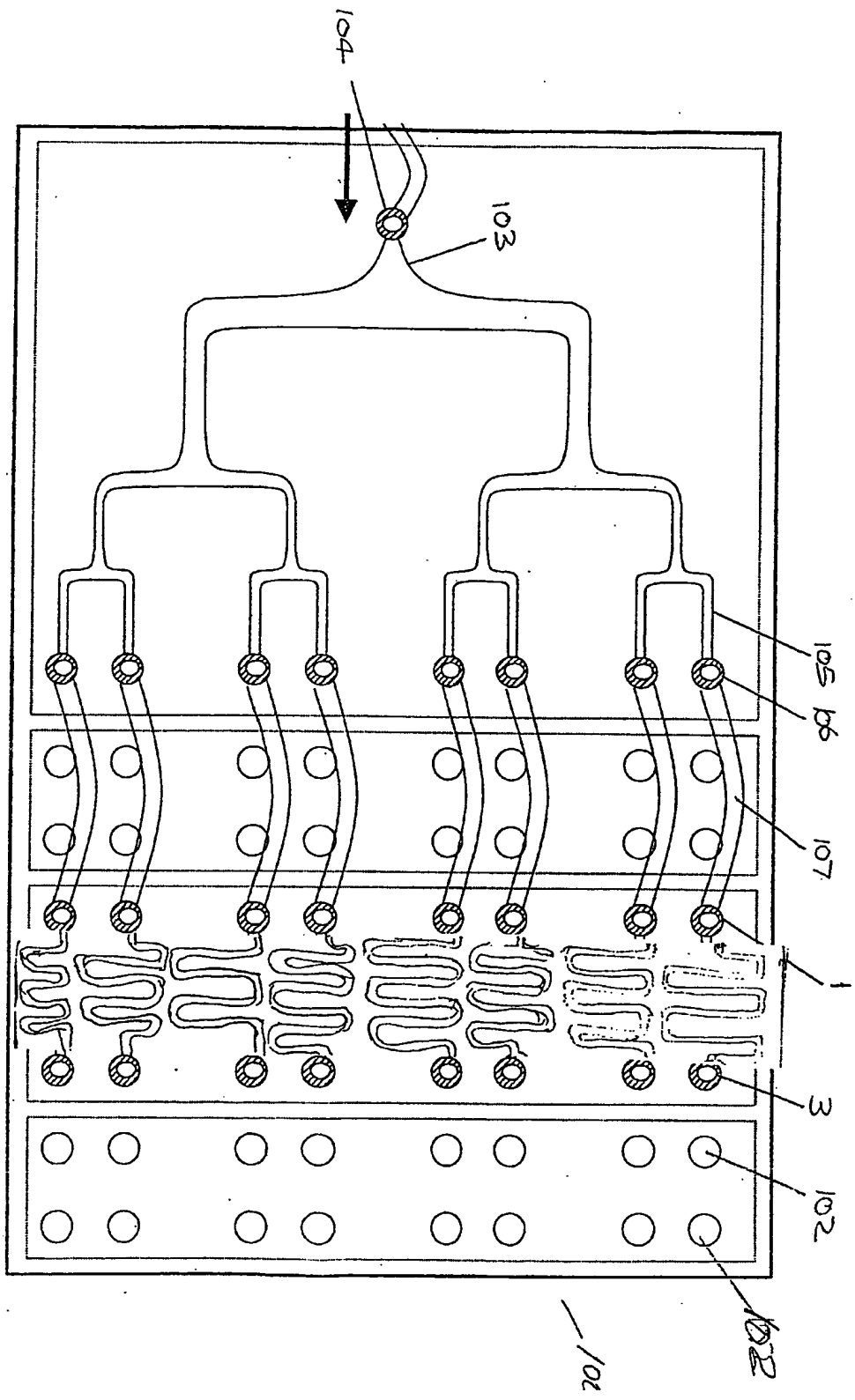

Fig. 30

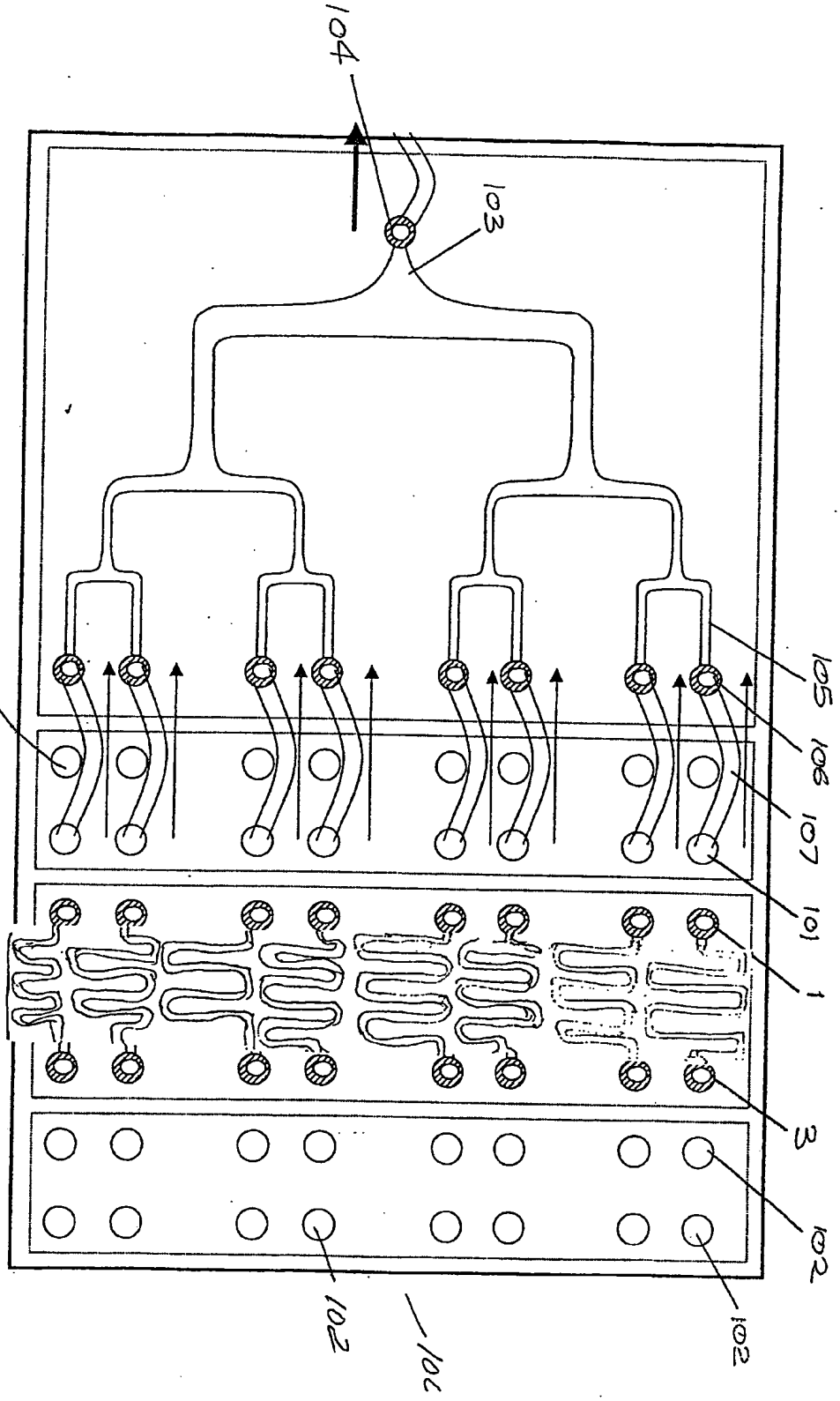

Fig.31.

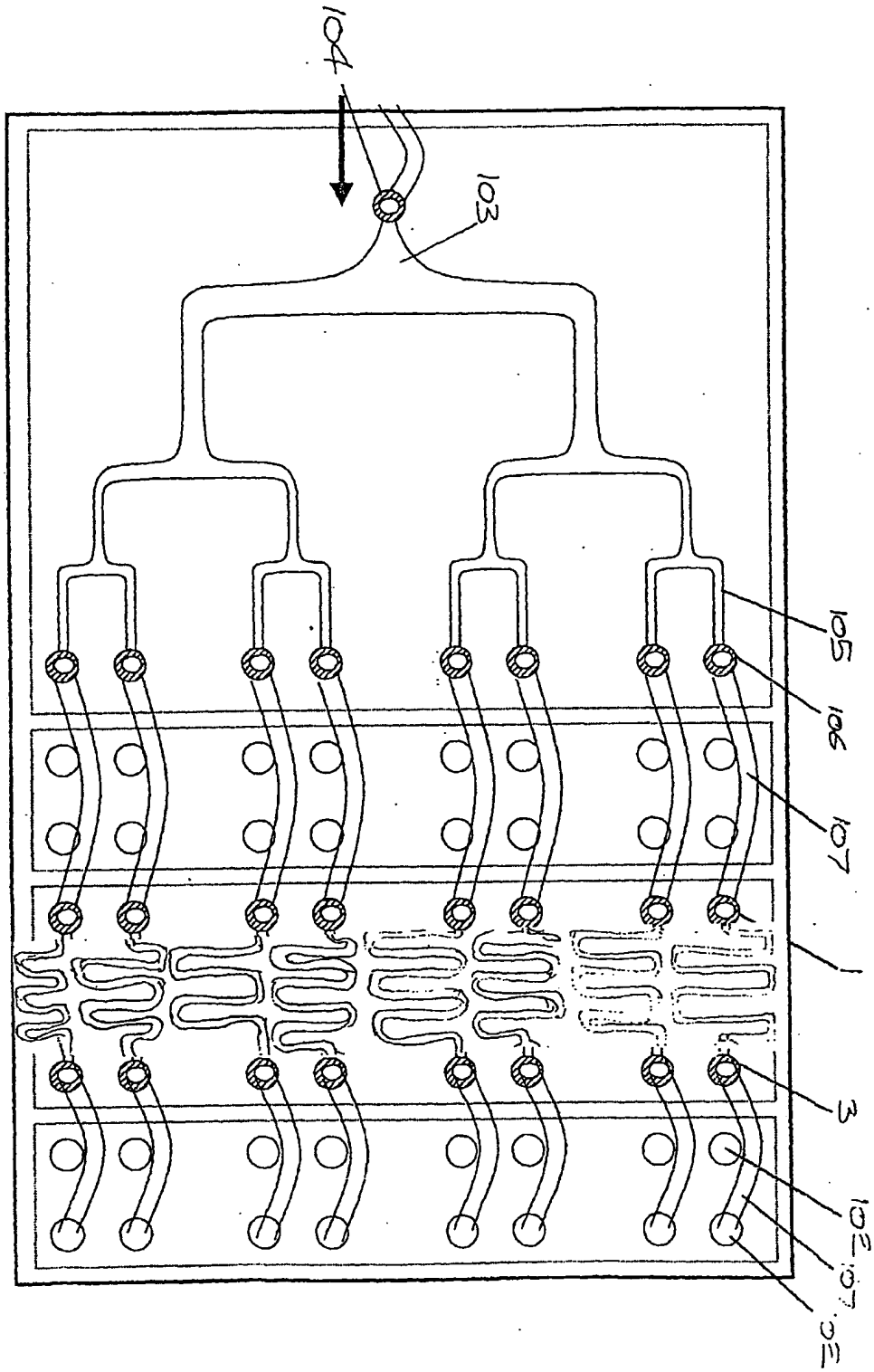

Fig. 22